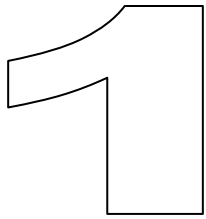
Regulation of multidrug resistance in Lactococcus lactis

Agustiandari, Herfita

Chapter



General introduction

THE RISE OF THE SUPERBUGS

Just about all organisms maintain close physical contact with other organisms in a changeable and dynamic ecosystem. Networking is important to cope with sudden environmental changes and it is beneficial for survival. Changing conditions and competition amongst organisms for essential nutrients, together with humans' interference in the global biosphere have lead to rapid evolutionary changes in all organisms involved. The first introduction of pesticides and antibiotics during the industrialization era in 1940's significantly increased the quality of humans' life as it provided protection against pests and infections caused by pathogenic bacteria (121). However, due to the extensive use of antibiotics and other toxic compounds, bacteria quickly developed numerous ways to gain resistance against these molecules. The ability of bacteria to be resistant to several chemically unrelated drugs is termed the multidrug resistance (MDR) phenomenon. Bacterial MDR was thought to arise via natural selection involving spontaneous mutation(s) in the genome to execute novel activities against a range of antibiotics. This resistant trait is generally passed on to the next generation and quickly leads to a fully resistant colony. It is important to note that bacteria did not evolve a specific response towards the presence of antibiotics. Rather, they became resistant via the mobilization and/or modification of pre-existing defense mechanisms that allow them to cope with the unfavorable environmental conditions (157).

One specific example of drug resistance is the re-emergence of the multidrug resistant tuberculosis bacterial strain (MDR-TB) which emphasizes the need for a new strategy and control of infectious disease since treatment with conventional drugs such as rifampicin and isoniazid have failed frequently (63). The first report on MDR-TB from 2005 showed that this organism is not only resistant to the previously used drugs but also to three out of six classes of second line antitubercular drugs like aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine and para-aminosalycilic (63). This new strain is also known as the extensively drug resistance tuberculosis (XDR-TB) that has emerged due to the improper use of antibiotics during treatments of tuberculosis (60). Another concerning example is the methicilin-resistant *Staphylococcus aureus* (MRSA), a major cause of nosocomial infections that can be acquired in hospitals. This organism readily takes up new plasmids, transposons, and its genome easily undergoes mutations. Its resistance to the blockbuster antibiotic vancomycin has lead to the emergence of a new strain known as VRSA (vancomycin resistant *S. aureus*) (1).

Molecular studies on multidrug resistance intensified after the discovery of P-glycoprotein (P-gp) in mammalian cancer cells (157). Cells that overexpressed

P-gp survived the cytotoxic effects of anti cancer drugs during therapy, and became cross resistant to a wide range of drugs from different classes (157). Shortly after the discovery of P-gp, bacterial resistance to antibiotics became apparent (75,105) as well as for instance the emergence of resistant insects to pesticides (12,41). Multidrug resistant bacteria were first observed in 1960s (176), followed by the discovery of cross resistant tumors and cell lines which occurred after the introduction of combined chemotherapy (19,67). P-gp is a transport protein that expels drugs from the cell thus, protects the cells against the adverse effects of these drugs.

MULTIDRUG RESISTANCE IN BACTERIA

Several mechanisms exist to gain resistance to toxic compounds. They are based on drug degradation via enzymatic reactions, an alteration of the drug target, the prevention of drug entry, and finally the active drug expulsions from the cell. For instance, some bacteria produce β-lactamases that catalyze the hydrolysis of the β-lactam ring of penicillin (162) while chloroamphenicol transferase modifies aminoglycosides into inactive entities (11). Drug target alteration prevents or reduces the binding of the drug. For instance, some amino acid mutations in penicillin binding proteins (PBPs) prevent the irreversible binding of penicillin which subsequently renders peptidoglycan biosynthesis penicillin-insensitive (162). In tetracycline resistant bacteria, the ribosomes often undergo a covalent modification that prevents the binding of tetracycline (160). However, these mechanisms are specific only to certain classes of antibiotics and usually do not results in multidrug resistance. Bacteria contain a cytoplasmic membrane that function as a barrier that separates the inner and the outer part of the cell. This barrier also prevents the rapid entry of small molecules like drug(s). Gram-negative bacteria contain an additional outer membrane that consists of phospholipids and lipopolysaccharides (LPS) which exhibits a reduced permeability towards lipophilic drugs. Gram-positive bacteria have no outer membrane but instead a thick peptidoglycan layer that allows diffusion of small molecules (108). Mycobacteria on the other hand, contain a thick extracellular layer of mycolic acids that is highly impermeable for drugs. Both Gram-negative bacteria and mycobacteria can decrease their membrane permeability via the loss of porins (107). However, these barriers do not counteract the toxicity effect of the drugs once they enter the cells. Therefore, cells need another line of defense and one of the most common mechanisms is the active drug extrusion from the cell by transporter proteins embedded in the cytoplasmic membrane. Drug extrusion is an

energy dependent phenomenon and a growing number of membrane proteins have been described to function as multidrug resistance efflux proteins. The active extrusion of drugs appears to be the main mechanism of MDR and SDR (specific drug resistance) in bacteria (132).

MULTIDRUG RESISTANCE TRANSPORTERS IN BACTERIA

Tetracycline resistance in both Gram-positive and -negative bacteria is due to the expression of transporters that mediate tetracycline efflux (28). These proteins belong to a large family of MDR transporters that are equipped with a broad substrate specificity of many chemically unrelated compounds (123,132,141). Based on their bioenergetic mechanisms and structural differences, bacterial MDR transporters can be divided into two major classes, i.e., primary and secondary transporters. Some bacteria mostly rely on the primary transporters for drug resistance, whereas others utilize the secondary transporters (125,126). Primary MDR transporters belong to the ABC superfamily and use the free energy derived from ATP hydrolysis to extrude toxic compounds from the cell (55). ABC transporters involved not only in drug export but also in a variety of other cellular activities such as peptide export, uptake of nutrients, and also in transport-unrelated functions such as DNA repair, translation, and regulation in gene expression (24,31,55). The proteins that are involved in the transport unrelated functions utilize the universally conserved ATP-binding cassette for their catalytic activities and lack the membrane domain. ABC transporters are membrane proteins with a typical organization, i.e., two nucleotide binding domains (NBD) where ATP hydrolysis take place, and two transmembrane domains (TMD) responsible for substrates recognition and transport (55). Each TMD consists of six membrane spanning α-helical domains with a low amino acid sequence identity among the group of ABC transporters. The two TMDs may exist as a single fused polypeptide chain that associates with a heterodimeric, homodimeric or even two fused NBDs. The MDR transporters are usually single polypeptides that each comprise of two TMDs and NBDs. Some MDR transports are homo- or heterdimeric proteins that built from subunits consisting of a TMD fused to the NBD. The secondary multidrug transporter mediate drugs extrusion in a coupled exchange with protons (or sodium ions) also known as drug/H+ or drug/Na+ antiporters (132). Secondary transporters form the largest group of known extrusion systems in bacteria comprising of four subdivisions, i.e., the major facilitator superfamily (MFS), the small multidrug resistance family (SMR), the resistance-nodulation-cell division family (RND), and the multidrug and toxic compound extrusion (MATE) family.

These MDR transporters share little homology amongst each other but they may recognize a similar range of substrates.

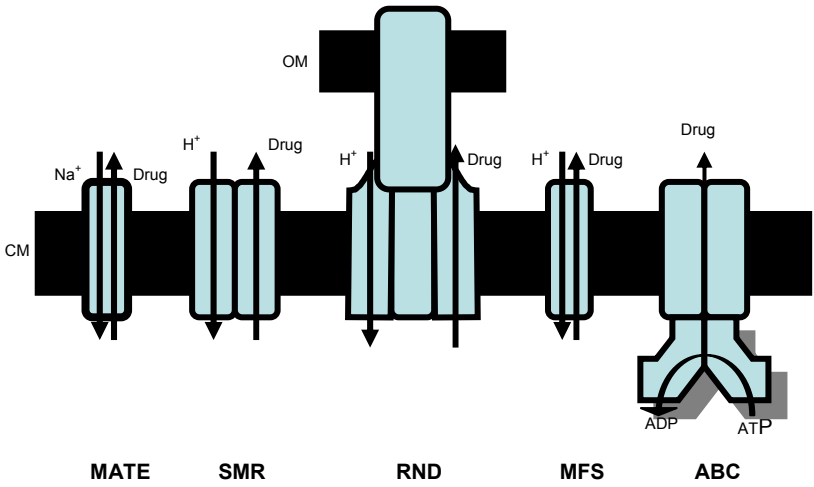


Figure 1 Distribution of MDR pumps in Gram positive and negative bacteria. Primary transporters utilize the energy derived from ATP hydrolysis to secrete drugs out of the cells. They belong to the ATP-Binding Cassette (ABC) superfamily and function either as a homo- or hetero dimeric transporters. To date, many of the ABC type transporters involved in MDR are mostly found in the Gram positive bacteria. Secondary transporters are driven by the proton motive force (PMF), and catalyse drug export by H^+/Na^+ antiporter: Transporters of the Major Facilitator Superfamily (MFS) function as monomers, while trasnporters of the Small Multidrug Resistance family (SMR) function as either a homo or heterodimers. The Resistance-Nodulation-cell Division (RND) transporters consist of a tripartite system and functions as trimers. RND transporters are mainly found in the Gram negative bacteria and require accessories protein such as an outer membrane protein (OMP) and membrane fusion protein (MFP). Another member of the secondary transporters belongs to the MATE (Multi Antimicrobial Extrusion) family that use the sodium motive force.

PHYSIOLOGICAL FUNCTIONS OF MULTIDRUG RESISTANCE IN BACTERIA

The exact physiological function of most bacterial MDR transporters is unknown, but often they are involved in the defense mechanisms against specific class of chemical compounds. The MDR phenotype is often associated with an enhanced expression level of these transporters or by mutations in the structural gene. For instance, the expression of the *B. subtilis* MDR transporter Blt results in an

increase efflux of spermidine in the medium (188). The *blt* gene is co transcribed with the *bltD* gene which encodes a spermidine acetyltransferase which catalyzes a key step in spermidine degradation. Reserpine, an antagonist of MDR transporters inhibits spermidine efflux by Blt (187) showing the close functional relationship of Blt with MDR transporters. The ability of some MDR transporters to recognize lipids or fluorescence lipid derivatives, and to transport detergent (133,185), bile salts (84,168), organic solvents (184), and ionophores (37,156), might indicate a natural functions in phospholipid transport or of lipid linked precursor of peptidoglycan (23,59,86,177,191). Various antibiotics that appear to be the substrates of the MexAB-OprM transporter of *Pseudomonas aeruginosa* are secondary metabolites that result from the aromatic amino acid biosynthesis pathway (129). For instance, the presence of iron in the medium significantly increased the expression of MexAB-OprM causing the efflux of peptide pyoverdine that is involved in iron uptake (130). This finding suggests a physiological role of MDR-like transporters in secondary metabolite excretion.

REGULATION OF MULTIDRUG RESISTANCE IN BACTERIA

Bacterial MDR can be either intrinsic or acquired. The basal expression of MDR pumps in the wild-type only suffices to provide a certain level of protection. At a higher drug concentration, resistance can be acquired via several mechanisms: (i) amplification and mutations of the MDR transporters causing changes in the expression level (106) or activity (70); (ii) mutations in the regulatory genes causing constitutive expression of the MDR transporters (2), and, (iii) resistance gene transfer among cells via transposons and plasmids (68*)* (For general review of mechanisms of bacterial resistance see refs (8,49)). Regulatory proteins respond to changes in the environment and trigger necessary cellular modifications at the transcriptional, translational or protein levels. Transcriptional regulators often comprise two-domains, i.e., the DNA binding domain and a ligand binding domain. Other regulatory mechanisms belong to the so-called two-component regulatory systems. Herein, the membrane-linked kinase acts as a sensor and in the presence of the inducer, the kinase will phosphorylate the DNA binding protein, which subsequently modulates the transcription of the gene(s) of interest by binding to the cognate promoter (135) (Table 1).

Although a lot of efforts have been made to understand the mechanisms of drug transporters in bacteria, the hydrophobic nature of these proteins make them intrinsically difficult to crystallize in order to obtain structural information. A few high resolution structures of MDR transporters are now available for instance

Sav1866 (32) however, the elucidation of the exact mechanism of action and the molecular basis of drug recognition and specificity remains a challenge. On the other hand, the structural and functional analysis of the regulatory pathways that govern the expression of these MDR transporters progresses rapidly. The expression level of many MDR transporters is closely controlled by inducers that are also substrates for the transporters. Many of the MDR transporters are subject for regulation by the transcriptional regulatory proteins. Gene regulation is crucial as an uncontrolled expression of the MDR transporter might be toxic to the cells. Transcriptional regulators are cytoplasmic proteins that can act as activators or repressors that influence transcription of target gene(s) at local or global level (46). Transcriptional regulators can also undergo autoregulation (2,56). A general mechanism for resistance development upon the exposure of cells to drugs or antibiotics is the immediate up-regulation of the low-expressed MDR transporters. For instance, the local transcriptional activator BmrR of *Bacillus sub*tilis (3) and the transcriptional repressor QacR of *Staphylococcus aureus* (43) enhance the expression of the MDR transporters Bmr and QacA through the binding of chemically unrelated cationic drugs like tetraphenylphosphonium (TPP^+) and Rhodamine 6G, respectively. The expression of *E. coli* RND transporter AcrAB is under the regulatory control by the global activators MarA, Rob, and SoxS (6). Derepression of *mexAB-oprM* operon in *nalB*-type mutant bearing mutation in *mexR* causes resistance phenotype to fluoroquinolones, chloroamphenicol, and β-lactams antibiotics (131,142). The expression level of *mexAB-oprM* during the exponential growth phase is being control by the local transcriptional repressor MexR (36). Interestingly, in the early stationary phase of growth, a higher expression of *mexAB-oprM* is being modulated by the accumulation of quorum sensing autoinducer C4-HSL in the medium, and not by MexR (143).

The structural studies of transcriptional regulators of MDR transporters are of particular interest as it may provide an insight in the molecular basis of drug recognition. They often interact with the similar panel of substrates as recognized by the MDR transporter. A typical DNA binding domain of bacterial transcriptional regulators comprises an α-helix-turn-helix (HTH) motif that may be organized in different structural environments such as three-helix bundles and winged helix motifs (120). About 95% of all bacterial transcriptional factors use the HTH motif to bind to their target DNA (25,38,50,51,127,140). MDR related transcriptional regulators belong to one of the following families: AraC, MarR, MerR, and TetR. The distinction is based on the high level of similarity of the N-terminus DNA binding domain that constitutes about one third of the polypeptide. Recent findings indicate some of the MDR-related transcriptional regulators belong to the PadR family for instance, the LadR of *Listeria monocytogenes* and LmrR of

Lactococcus lactis. PadR regulatory proteins are involved in the regulation of detoxification pathways in bacteria such as phenolic acid metabolism. The following section describes in detail mechanistic and functional aspects of MDR-related transcriptional regulators

QacR regulates the expression of the MDR transporter QacA/B in *Staphylococcus aureus*

QacA and QacB are MDR transporters of *S. aureus.* These two nearly homologous genes belong to the MFS family. In a proton motive force (PMF)-dependent manner, they extrude a wide range of mono- or bivalent cationic lipophilic substrate from the cell. The identified substrate range encompasses of 12 different chemical groups (79,102,103,122) which mostly concern positively charged lipophilic compounds that are able to pass the cytoplasmic membrane, and accumulate in response to the transmembrane electrical potential, $\Delta\psi$ (negative inside). A gene located upstream of *qacA* termed *qacR* specifies a trans-acting transcriptional repressor of the *qacAB* genes. These genes are found on the multi resistance plasmids such as pSK1 present in clinical isolates (73,124,166,167). The QacR protein is a product of a divergently transcribed ORF that binds specifically to the promoter region of *qacA* thereby repressing its transcription (43). QacR belongs to the TetR/CamR family of transcriptional regulators that share a high degree of homology in their N-terminal DNA binding domains (139). In the absence of QacA substrates, QacR binds to a region of dyad symmetry (IR1) consisting of 15 base-pairs with each half site separated by a 6 base pair spacer sequence (43). Binding of QacA substrates to QacR causes the derepression of *qacA* transcription. This event leads to the overexpression of the QacA transporter and thus, to MDR in *S. aureus*. The direct interaction between QacR to structurally unrelated QacA substrates like benzalkonium, dequalinium, ethidium bromide, chlorhexidine and rhodamine 6G induces conformational changes in QacR which results in a reduction of its binding affinity to the IR1 operator DNA (43). Interestingly, plant alkaloids such as berberine, an amphipathic cationic compound, induces both the expression of the plasmid encoded *qacA* and chromosomally encoded *norA* genes, which both encoded for MDR transporters (47). Therefore, the QacA-QacR system provides protection against compounds produced by plants and naturally derived cationic and hydrophobic antimicrobial agents. The presence of the *qacA-qacR* locus on multi resistance plasmids in clinical *S. aureus* strain suggests that the system also functions to protect cells against synthetic man-made drugs (73). QacA shows a much greater substrate range as compare to QacB. Thus

QacA may have evolved from QacB to provide resistance against clinical drugs (124).

DNA binding: QacR binds to the palindromic sequence at the IR1 DNA operator site as dimers. Upon addition of substrates, the four DNA bound QacR molecules dissociate as dimers (44). This observation shows that QacR does not self assemble into tetramers, while the two dimers appear to have no direct contact with each other in the DNA bound state (44). QacR adopts a somewhat different DNA binding mode than TetR (153). The structure of the QacR: DNA complex shows that the two QacR dimers bind to the extended 28 base pair IR1 operator on the opposite sites, in which the two HTH motifs from each dimer make a contact with the DNA major groove (153). QacR comprises nine α-helices (153). The first three helices form the three helix bundle DNA binding domain of the HTH motif (α2 and α3). DNA recognition occurs in the α3 helix. The DNA binding domain of QacR is highly homologous to TetR but the N-terminus of α1 in QacR makes contact with the phosphate backbone in a different orientation than the corresponding helix in TetR (153). The dimerization domain/substrate binding site of QacR comprises α-helices 4 to 9 that show a low homology to the corresponding domain of TetR. The C-terminal anti-parallel α-helices from each dimer form a four helix dimerization region (153).

The QacR dimers bind cooperatively to the operator DNA which is an exceptional feature among members of the TetR transcriptional regulators (153). Gel filtration and light scattering studies are consistent with a cooperative binding of two QacR dimers to the IR1 DNA (153). Because of the large distance of > 5 Å between each dimer, this cooperative binding mode does not seem to involve protein-protein interactions (153). Rather the structure and adopted conformational changes in the DNA inflicted by the bound QacR causes such a cooperative binding behavior (153). There are 16 bases and 44 phosphate contacts with the IR1 site generated by the binding of the two QacR dimers. Each monomer contacts the DNA differently; one distal and one more proximal to the dyad, each recognizing particular bases (153). The removal of two base pairs from the wild-type six base pair spacer region abolishes the binding of QacR, confirming that spacing is important for proper binding to the IR1 operator. A common feature of the TetR/CamR members of transcriptional regulators is their short recognition helix that insert deeply into the floor of the DNA major groove. One notable difference between TetR and QacR concerns their DNA binding mechanism. Upon binding to the DNA, TetR induces a bending of 17° of the DNA towards the protein that optimizes the HTH interaction within each DNA half-site including a widening of the DNA major groove. On the other hand, the entire IR1 operator widens upon binding of the two QacR dimers resulting in a minor bending of the DNA by only

3° (153). The HTH motif of TetR makes identical contacts to each symmetry DNA half site which is a common feature of dimer binding to a palindromic sequence in protein-DNA interactions. However, each HTH motif of the QacR dimer makes contacts at a non palindromic site on the operator DNA and thus it is not symmetrical.

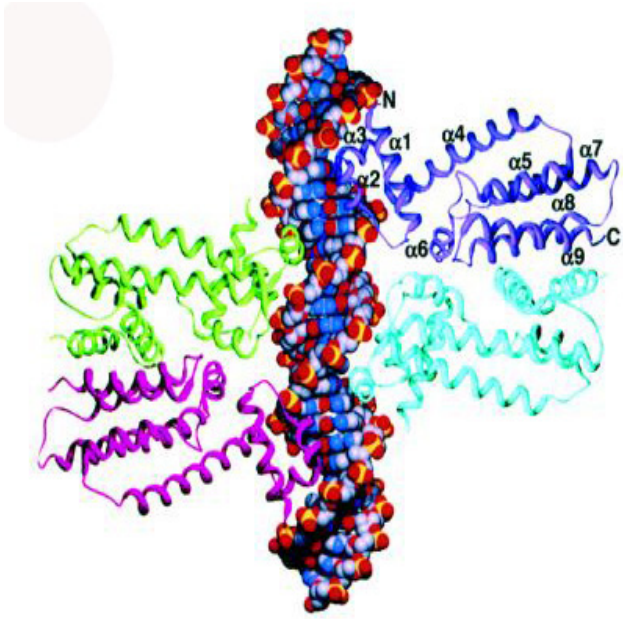


Figure 2. The crystal structure of QacR: DNA complex solved at 2.90 Å resolution. The four identical monomers of QacR form two functional dimers and bind to the symmetrical version of a 28 bp IR1 operator site of *qacA* in the absence of drug molecules. Reproduced from reference 153 with permission.

Drug binding: QacR shows an unusual feature of overlapping mini pockets within its large hydrophobic drug binding site (151). These mini pockets can bind different substrates, and serves as a model for multi-site binding allowing interactions with a range of structurally distinct drugs. Structural studies on both AcrB transporter that contain such mini pockets as well as the transcriptional regulator QacR revealed interactions between these mini pockets and displayed no clear boundaries (151). Moreover, a low resolution structure of the MDR transporter AcrB was shown to bind four different drugs simultaneously (151). TtgR of *Pseudomonas putida* is structurally related to QacR. Unlike QacR that

binds to a variety of positively charged substances, TtgR binds negatively charged toxins due to the presence of polar residues in its hydrophobic binding pocket. TtgR also bind a broad range of naturally occurring antimicrobial compounds such as phloretin, quercetin and naringenin (10). Interestingly, the QacR dimer always binds only one ligand whereas the TtgR dimer has been showed to bind up to three molecules of phloretin; two molecules at low- and one molecule at the high affinity binding sites. The multidrug binding pocket of TtgR is larger in comparison to QacR and posses two overlapping binding sites; a highly hydrophobic general site that binds compounds such as tetracycline, chloroamphenicol, quercetin, and naringenin (one ligand per TtgR dimer), and a polar site for high affinity phloretin binding pocket (10).

QacR exhibits positive or negative cooperativity between certain drug classes as well as non cooperative and uncooperative interaction with others (151). QacR binds both mono- and bivalent cationic drugs. Monovalent drugs like rhodamine 6G and ethidium bromide bind non simultaneously to different but overlapping regions of the extended QacR ligand binding pocket (152). Because of the presence of flexible carbon atom linker in the bivalent dequalinium, this molecule can interact with both binding sites of QacR. Rhodamine 6G and ethidium bromide bind through their ring system (152). Hence, it appears that QacR also can bind two (or more) drug simultaneously at overlapping binding sites.

Proflavin and rhodamine 6G bind QacR at overlapping sites. Upon binding, proflavin stacks with the side chains of Trp61 and Tyr93, whereas Glu57 and Glu58 help to neutralize the positive charge and sandwich the proflavin ring system in place (151). In the QacR dimer, proflavin binds at the same site as rhodamine 6G and both compete for binding (151). Rhodamine 6G and ethidium bromide bind to different but overlapping binding sites. These drugs do not bind to the QacR dimer simultaneously possibly because of steric hindrance (151). The structure of QacR in complex with both proflavin and ethidium bromide was solved at 2.96 Å resolution (151). Binding of both drugs simultaneously did not result in additional structural changes of the QacR dimer (151). Upon dual drug binding to the QacR dimer, structural comparison between the QacR: proflavin: ethidium bromide and QacR: ethidium bromide binary complexes showed the ethidium bromide molecule had shifted to another pocket to avoid clashing with proflavin. This adjustment offers a closest ring-to-ring approach of 4.0 Å engaging ethidium bromide in a favorable van der Waals interaction with proflavin while still preserving the hydrophobic and aromatic stacking interactions with the QacR protein (151). Aromatic residues play a critical role in drug binding. They are needed for the structural organization of the multidrug binding pocket and in the

drug-binding induced conformational transitions allowing the drug to slide into the binding pocket while still maintaining stacking interaction (151).

Regulation of drug resistance transporters Bmr and Blt in *Bacillus subtilis*

Bmr and Blt are two MDR transporters of *B. subtilis*. They shares up to 51% amino acids sequence homology and when overexpressed they are involved in the export of a similar range of drugs (4). Unlike *bmr*, *blt* is not transcribed in the wild-type cells. Likewise, disruption of the *bmr* gene but not of *blt* causes cells to be more susceptible to toxic drugs (4). Bmr is involved in the export of a diverse range of compounds that are lipophilic, monovalent and cationic (3,178). Most of the substrates are also substrates of P-gp and the transport activity of Bmr can be inhibited by reserpine and verapamil. Interestingly, cells challenged with rhodamine did not show neither the up regulation of *blt* transcription nor the interaction of this compound with the transcriptional activator BltR, whereas *in vitro*, rhodamine is a substrate for the Blt transporter (4). This suggests that the substrates binding domains of BmrR and BltR are functionally different.

DNA binding: The level of expression of *bmr* and *blt* is under a control of transcriptional activators BmrR and BltR, respectively (4). These proteins belong to the MerR family of transcriptional regulators. Although BmrR and BltR are very homologous in their N-terminal DNA binding domains, their C-terminus drug(s) binding site show little sequence homology (4). Unlike BmrR, the identity of the ligand(s) that bind BltR is still unclear although is it clear what substrates are transported by Blt. The *bmr* and *blt* genes localize in different operons. The *bmr* promoter exclusively controls the expression of *bmr* while the *blt* promoter controls the expression of two genes, *blt* and *bltD*. BltD is highly homologous to an acetyltransferase that could implicate a metabolism-associated function of the Blt transporter (4).

Transcriptional activation of both *bmr* and *blt* genes is subject for regulation by the global activator Mta (13). A truncate of Mta that comprises only the N-terminal DNA binding domain (MtaN) binds both the promoter regions of *bmr* and *blt* at exactly the same nucleotide sequences that are bound by BmrR and BltR, respectively (13). MtaN comprises the winged HTH motif that is structurally related to the same region of BmrR. However, the α5 dimerization helix and the first wing of the DNA binding domain of MtaN are in different orientations as compared to the corresponding regions in BmrR suggesting these two protein interact with DNA in a distinct manner (13). With respect to the drug binding domain, Mta is homologous to the global regulatory protein of multidrug resistance TipA in *Streptomyces lividans* (13,58). Therefore, *bmr* and *blt* expression by Mta

may be at the level of global control, and their expression can be effected by stresses induced by means of the presence of toxic compounds or peptides in the medium. The *S. aureus* NorA protein is homologous to the *B. subtilis* Bmr and Blt (4). Likewise, NorA provides resistance to the cells to a similar panel of drugs as transported by BmrE. However, little is known about the regulation of *norA* expression.

The BmrR dimer directly interacts with drugs. Ligand-bound BmrR binds to the inverted repeats located between the -35 and -10 region of *bmr* promoter region. The -35 and -10 consensus motifs are separated by a 19 base pair spacer sequence which is quite long as compared to the typically observed 16-17 base pairs (4). The long spacer sequence together with the location of the -35 and -10 region on the other side of the DNA helix are necessary to prevent activation of *bmr* transcription by RNA polymerase in the absence of drugs (4). The constitutive drug resistance phenotype of selected *B. subtilis* strains is caused by a 2 base pair deletion in the spacer region of *bmr* promoter causing continuous overexpression of Bmr (54). Constitutive expression of *blt* was observed in *B. subtilis* 168ACF that carries the *acfA* mutation, i.e., a 1 base pair deletion in the spacer region of the *blt* promoter (4).

BmrR belongs to the winged-helix superfamily of a four-helix-bundle and a three stranded antiparallel β sheets (39). The topology of BmrR HTH motif corresponds to β1, α1, α2 (recognition helix), W1 (sheets β2 and β3), W2 (α3 and α4) with α5 function as a linker connecting the DNA and substrate binding domains (54). The DNA binding elements of BmrR are responsible in making contacts with the *bmr* promoter while still promoting the favorable DNA conformation for binding of RNA polymerase (54). The residues Ser41, Tyr42, and Arg43 in W1 are necessary for DNA binding through van der Waals interactions and hydrogen bonding. Moreover, Arg43 together with a glutamate, aspartate or glutamine at position 26 are conserved amongst MerR family members indicating that W1 is an important DNA binding element (54). In contrast to other winged-helix proteins, α2 and α3 of W2 of BmrR are part of the HTH motif rather than forming a loop (54).

The structure of the ternary BmrR:TPP^{+}:DNA complex shows a remarkable twisting of the promoter region with a reorientation of the -35 and -10 region to allow RNA polymerase to bind and initiate transcription (54). This mechanism involves base pairing disruption of 2 residues in the center of the pseudodyad of the *bmr* promoter (54). The A-T base pair at +1 position breaks causing the adenine and thymine to slide away from each other. On the other hand, the A-T base pair at the -1 position is able to form miss-pairing interaction regardless of the misplacement (54). The resulting DNA distortion causes a

"bunches up" in the middle of the operator region and a space shortening equivalent to 2 base pairs (54). Residues Tyr24, Tyr25, Lys60, and Lys66 of BmrR stabilize the base pair distortion via contact with the DNA back bone (54).

Drug binding: The binding of ligands to BmrR was previously studied using the isolated C-terminal substrate binding domain (BRC) (87). This domain can be readily expressed, dimerized, and able to bind two drugs per dimer (87). This finding is consistent with the BmrR:drug:DNA complex which shows one drug molecule bound per BmrR monomer (54). There is no obvious binding pocket or cavity found in apo BRC structure (192). However, the BRC:TPP^+ structure revealed the presence of a charged residue (Glu134) buried in the core of the protein but positioned at the base of the binding site (192). Ligand binding occur via electrostatic interaction between the negative charge of the carboxylate group of Glu134 with the positively charged substrates like TPP^+ or Rhodamine 6G (192). Glu21 initiates the entry of TPP^+ into the internal binding site of BRC. This residue is located proximal to α2 and creates a negative patch that attracts cationic compounds (54). Binding of molecules will take place only via the unfolding of α2 (54). The BRC Glu134Ala mutant shows reduced binding affinity to five out of six BmrR ligands (178). This suggests the presence of a multi variant binding site that can accommodate a diverse range of chemically unrelated compounds. Structural comparison between the apo BRC and BRC:TPP+ showed no major conformational changes which suggests the unwinding of α6 in BmrR may act as a signal during drug induced transcriptional activation (192).

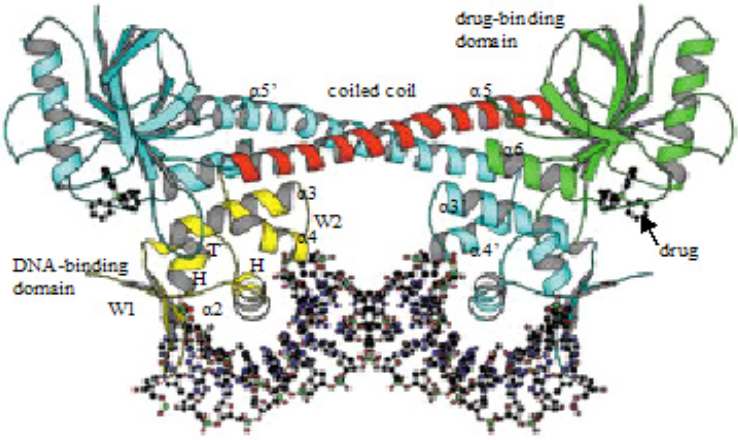


Figure 3. A structural overview of the BmrR dimer with bound DNA and drug TPP. The N-terminus is responsible for the DNA binding domain and it is directly linked to the C-terminus which comprised two-third of the protein responsible for the ligand binding domain indicated by a long helix α5. Reproduced from reference 54 with permission.

TetR regulates the expression of the specific tetracycline MDR pump: TetA

The overexpression of TetA transporter results in resistance to tetracycline. TetA belongs to the MFS and specifies an antiporter that mediates the extrusion of tetracycline in complex with a divalent metal cation e.g. Mg^{2+} in exchange for protons. Unlike Gram-positive bacteria, expression of *tetA* in Gram-negative bacteria is controlled by the product of a divergently transcribed gene, the *tetR* repressor (56). TetR proteins can be classified into 8 subfamilies, i.e., TetR A to E, G, H, and J (144). TetR proteins are transcriptional regulators that control the transcription of various genes associated with antibiotic biosynthesis, osmotic stress, multidrug resistance, and pathogenicity in both Gram-positive and -negative bacteria (135).

The best characterized TetR protein is encoded by the *Tn10* gene. TetR(B) binds to the overlapping sequences of *tetA*(B) and two divergent *tetR*(B) promoter regions (56). Homodimeric TetR binds non-cooperatively to the two adjacent inverted repeats known as the *tet* operators O_1 and O_2 that overlap with the *tetA*(B) and *tetR*(B) operator DNA region (98). Binding of TetR in the presence of the tetracycline-Mg^{2+} complex induces conformational changes in TetR, whereupon derepression of both *tetA* and *tetR* genes occur which expel tetracycline from the cell (57). TetR shows a higher binding affinity for tetracycline-Mg^{2+} complexes than the drug target, the ribosomes. This ensures a full protection to the cells via TetA overexpression (56). TetR binding affinity to O_2 is four folds higher than to the O_1 sequence (69).The binding of TetR to O_1 results in a complete block of transcription of both the *tetA* and *tetR* genes, whereas down regulation of *tetA*(B) but not *tetR*(B) gene is being observed when TetR binds to the O_2 site only (98). When the low amount of repressor-operator bound complex is detected, the synthesis of *tetR*(B) but not *tetA*(B) is being activated. In addition, the uncontrolled expression of the integral membrane protein TetA is lethal to the cells as nonspecific cation transport causes a collapse of the $\Delta\psi$ (35).

TetR(D) is organized as a dimer. The crystal structure of the TetR(D):*tetO* complex shows that each subunit comprises of 10 α-helices (117). α8 and α10 from each monomer form antiparallel helices that are part of the four helix bundle of the dimerization domain (57,146) and protein-protein interaction (145,150). The HTH motif of TetR comprises α2 and α3 while α1 stabilizes the HTH structure and α4 contributes to the formation of a hydrophobic center in the DNA binding domain that is critical for the binding of TetR to the *tet* operator (117). In addition, truncation at the N-terminal region of TetR abolished the binding to DNA (17). In the TetR(D) bound tetracycline-Mg^{2+} complex, both α3 and α3' were found to be apart by 39.6 Å. This space is sufficient to prevent binding of the repressor to the

DNA major grooves that are about 34 Å apart (57). TetR (D) binds to the 15 base pair fragment of the *tet* operator except for the central 3 base pairs. Under those conditions, α3 and α3' of the TetR dimer are separated by 36.6 Å while binding of the drug increases this gap by 3 Å (117). The TetR-DNA interface contains no water molecules unlike common protein-DNA interaction. Pro39 of the HTH motif plays an important role in establishing this particular contact in the TetR-DNA complex (53,117,180). The ligand induced conformational changes of TetR(B) and TetR(D) have been described in detail elsewhere (52,57,104,148,149,158). The TetR homodimer traps two tetracycline-Mg^{2+} molecules inside tunnels that are located in the center of the protein (57). Each of the binding tunnels consists of α-helices that are derived from both monomers; the first tunnel comprises α-helices 4 to 8 and 8' and 9' while the second one consists of α-helices 4' to 8' and 8 and 9. The structure of ligand-bound TetR shows a large opening adjacent to the C-terminus at α9 (and α9' from the other subunit) that was not observed with the apo TetR. This large opening functions as a drug entry gate (57,115). The tetracycline derivative glycylcycline contains a glycylamido substituent that causes steric hindrance thereby interfering with the TetR sliding door α9'. This ligand does not induce the depression of *tetA* (118). By means of a chelator, the Mg^{2+} can be removed from the TetR:tetracycline-Mg^{2+} complex, and the resulting TetR:tetracycline complex has almost a similar conformation as the apo TetR. This demonstrates that Mg^{2+} fulfils a critical role in the induction mechanism (116). Further details on the mechanism of TetR regulation can be found elsewhere (18,115-117,148,169-171,179).

The multiple antibiotic resistance (mar) regulon in *Escherichia coli*

E. coli is resistant to multiple compounds such as dyes, antimicrobial agents, detergents, fluoroquinolones, and numerous antibiotics including β-lactams, chloroamphenicol, erythromycin, and tetracycline. This resistance is caused by the overexpression of the tripartite drug efflux pump AcrAB-TolC (84,96,109,114,184). The physiological function of the AcrAB-TolC complex in *E. coli* is to transport stress related toxic compounds that are found in natural environments such as fatty acids and bile salts (84,109,168). The overexpression of the global regulators MarA, SoxS, and Rob modulates the expression of many of the *mar* regulon genes by binding to the marbox upstream of each promoter. The *mar* box is a 20 base pair nucleotide sequence motif located in the promoter region of the target genes such as the *acrAB* and *tolC* genes (6,113,182). Previous studies showed that MarA/SoxS/Rob autoregulate their own transcriptions and of each

other (91,99,100,111), suggesting the presence of additional binding sites within the *mar, sox* and *rob* promoters.

Mutations in AcrR, a divergently transcribed local repressor of *acrAB* operon cause the increased resistance to fluoroquinolones in clinical *E. coli* isolates (66,110,181). AcrR posses the typical HTH DNA binding motif and belongs to the TetR family of transcriptional regulators (83). AcrR represses both transcription of *acrR* and *acrAB* but it does not induce the expression of these genes under general stress conditions such as 4% ethanol, 0.5 M NaCl, or when cells enter the stationary phase (83). Transcription of these genes is linked to an unidentified regulatory protein (83). Thus, the expression of *acrAB* is primarily controlled by AcrR while its induction is modulated by MarA and other global regulators. Previous results indicate that the physiological function of AcrAB might relate to the export of non-freely diffusible quorum sensing molecules (134). *AcrAB* expression increases as the growth rate decreases and this might be linked to the accumulation of the quorum-sensing signals produced by the cells (83,136).

MarA belongs to the AraC family of transcriptional activators (64,65,91,137). The cellular level of MarA is controlled by the MarR repressor, the first gene of the *marRAB* operon. Both MarA and MarR bind to the DNA region *marO* that is located upstream of the *marR* gene and contains several regulatory binding sites surrounding the *marRAB* promoter (45). MarA binds to the marbox of a large number of *mar* regulon genes (6), and it also activates its own transcription. The MarA binding site is located 16 base pairs upstream of the -35 and -10 region of the *marRAB* operon (91,137). Over 60 chromosomal genes are differentially regulated in *E. coli* cells that constitutively express *marA* (14). In another study describing the inducible MarA expression system, an additional of 67 MarA-regulated genes were identified (128). MarA activates at least 40 different promoters of target genes (95), and also a gene with a marbox that diverges substantially from the consensus sequence (15). One example is the transcriptional activation of *micF* by MarA. This gene produces an antisense RNA that repress the expression of *ompF*, a gene codes for the outer membrane protein that allows passive diffusions of small molecules (34). Thus, reduced drug influx by OmpF combined with an active extrusion by AcrAB-TolC is a very efficient response mechanism controlled by MarA when cells are exposed to toxic molecules. A recent study also shows that MarA functions as the repressor of *rob* transcription in *E. c*oli (147).

MarA binds DNA as a monomeric protein. The DNA binding site lacks any inverted or direct repeats commonly characterized in bacterial regulatory sequences. Interestingly, MarA and its homolog SoxS posses no ligand binding site which make these proteins are considerably smaller in size as compared to the

members of the AraC family (94). The crystal structure of MarA:*marO* complex reveals an unusual feature of two HTH DNA binding motif that are connected by a long α-helix (90). A typical HTH binding motif can recognize only 6 base pairs out of the 11-12 base pairs of the operator sequence (161). This is the reason why most of the bacterial regulatory proteins need to dimerize to comprise two HTH motifs. Monomeric MarA is capable of binding to two successive DNA major grooves utilizing its two HTH domains. A previous study revealed that the N-terminal HTH domain of MarA contacts the marbox consensus sequences (42). A more detailed description of the MarA:DNA complex and the binding mechanism can be found elsewhere (30,42,89,90).

MarR repress the transcription of the *marRAB* operon by binding to *marO* at sequences different from the marbox that is recognized by MarA (91). MarR binds as a dimer to two site in *marO,* assigned as site I and II located downstream from the MarA binding site (92). To a certain extent, MarA and MarR compete for binding to *marO* (91). Site I overlaps the -35 and -10 region while site II is neither required for repression nor it is needed for binding of MarR to site I (92). The crystal structure of MarR has been solved at 2.3 Å showing that MarR posses the typical DNA binding domain of the winged-helix family (9). The α3 and α4 helices of MarR form the HTH motif with the β-sheets functioning as the "wings". Importance residues for DNA recognition by MarR are located in the α4 helix as mutations in this region abolish DNA binding (5). The structure of MarR is stabilized by a number of salt bridges (9,76). MarR binds antibiotics like tetracycline and chloroamphenicol, weak aromatic acids like salicylate, and other compounds such as the redox cycling molecules menadione and plumbagin (7,155). Salicylate binds to two sites on each subunit of the MarR dimer. These binding sites are located at the surface near to the DNA recognition helix α4 (9). Hence, the binding of the inducer disturbs MarR binding to *marO*, causing the depression of *marRAB,* and *mar* regulon activation via the synthesis of MarA (45). In addition, the MarA homologs SoxS and Rob bind to *marO* DNA and activate the expression of genes belong to the *mar* and *sox* regulon which contributed to the MDR phenotype of *E. coli*. In addition, the nucleoid-associated global regulatory protein known as FIS also bind to a region within *marO* upstream of the marbox. FIS stimulates the MarA, SoxS, and Rob mediated activation of transcription. Further information on SoxS, Rob and FIS can be found in references (64,65,83,93,94,101,112,114,137,138,165,172,184). Analogous *mar* regulatory networks are found in many bacterial species suggesting that these organisms carry closely related genes (29,62,74,97).

PadR, a new family of transcriptional regulator involved in multidrug resistance

LadR is a transcriptional regulator that controls the expression of the MDR transporter MdrL in *Listeria monocytogenes* (61). Overexpression of MdrL results in the rapid efflux of rhodamine 6G out of the cells. Interestingly, LadR belongs to the PadR family of transcriptional regulators, while most of the MDR specific transcriptional regulators belong to the MerR, AraC, or LysR family of activators or are members of the TetR, MarR, or LacI family of transcriptional repressors (46). PadR regulators are involved in regulating the expression of the phenolic acid decarboxylase (*pad*) gene(s) that detoxifies derivatives of phenolic acids such as ρ-coumaric, ferulic, and caffeic acids (48). Some organisms such as *Pseudomonas* strains (40,119) and *Acetinobacter calcoaceticus* (154) use these compounds as a sole source of carbon for growth. Based on phylogenetic analysis of related firmicutes, LadR forms an independent group in a large family of PadR regulators (PF03551). There are two distinct families of the *padR*-related genes: subfamily I of longer sequence of about 176 amino acids, and subfamily II of shorter sequence of about 110 amino acids (61). LadR is the product of divergently transcribed gene *ladR* that is located upstream of *mdrL*, and it is conserved in all sequenced *Listeria* genomes. LadR proteins of *L. monocytogenes, L. innocua, L. ivanovii,* and *L. grayi* are closely related to the PadR protein of *Pediococcus pentosaceus* (61). The intergenic region of *ladR-mdrL* is 166 base pairs long and contains two non overlapping putative -35 and -10 regions (61). The -35 region of the *mdrL* promoter contains an inverted repeat with a sequence that fits with the consensus motif of the PadR binding site, i.e., ATGT-8N-ACAT (61). LadR is a 176 amino acids long protein with a low sequence identity (24-29%) with PadR regulators from *Pediococcus pentosaceus* (16) and *Lactobacillus plantarum* (48). The PadR proteins from *P. pentosaceus* and *L. plantarum* negatively regulate the *padA* gene specifies the phenolic acid decarboxylase (16,48). LadR shares 25.5% homology to the repressor and co-activator AphA of *Vibrio cholerae* (33,71,72). AphA is a quorum sensing regulator that activates the virulence cascade of *V. cholerae* and works in conjunction with another regulator AphB. AphA is a repressor of the penicillin amidase activity coded by *pva* gene (71,72). The crystal structure of dimeric AphA (33) reveals a number of conserved residues that are also found in LadR (61). AphA has two domains; a globular N-terminal domain and a distinct C-terminal domain. The N-terminal domain of AphA adopts the winged-HTH motif similar to MarR (33). MdrL, QacA/B, and Bmr have a common ability to bind rhodamine 6G. A LadR-dependent regulation model for the expression of *mdrL* has been proposed. In the absence of rhodamine, apo LadR binds to the *mdrL* promoter

at the PadR box and repress the transcription of *mdrL* gene. In the presence of rhodamine, deactivation of LadR takes place, and the transcription of *mdrL* gene is initiated resulting in the overexpression of MdrL which in turn mediates the efflux of rhodamine from the cells. Since LadR is a newly identified member of PadR family, its exact physiological functions in both non pathogenic and pathogenic *Listeria* species remains to be determined.

LmrR regulates the expression of the major MDR ABC transporter LmrCD of *Lactococcus lactis*

The Gram-positive bacterium *Lactococcus lactis* is a non-pathogenic bacterium and widely used in the fermented food production. The genome of *L. lactis* contains about 40 genes that encode putative MDR transporters (21,183). By heterologous expression in drug-sensitive *E. coli* strains, the MDR transporters LmrA and LmrP have been implicated in the drug resistance phenotype of *L. lactis*. However, recent gene inactivation analysis suggests that the intrinsic multidrug resistance of *L. lactis* is due to the expression of the ABC transporter LmrCD. LmrC and LmrD are half-transporters that heterodimerize to form a functional MDR transporter (82). Overexpression of LmrCD is sufficient to protect cells against several hydrophobic drugs e.g. daunomycin, ethidium bromide, Hoechst 33342, and the fluorescence dye BCECF-AM (82). Moreover, LmrCD was recently being shown to transport bile acids as one of its natural substrates (190). Deletion of *lmrCD* in *L. lactis* NZ9000 renders the cells to be hyper sensitive to drugs such as Hoechst 33342, daunomycin, ethidium bromide, rhodamine 6G, and cholate. The drug resistant phenotype can be restored by the expression of *lmrCD* but not *lmrA* (81). Transcriptome analysis of four drug resistant strains of *L. lactis* adapted to increasing concentration of daunomycin, ethidium bromide, cholate and rhodamine 6G (20) revealed a similar response of the up regulation of *lmrC* and *lmrD*, and another gene termed *lmrR* (formerly *ydaF*) (81). The *lmrR* gene (*l*actococcal *m*ultidrug *r*esistance *r*egulator) is located upstream of the *lmrCD* genes. Nucleotide sequencing on *lmrR* in the four MDR strains showed frame shift and point mutations resulting in the production of non functional LmrR variants. The up regulation of *lmrR* in these MDR strains suggested an autoregulation mechanism of *lmrR* expression. Moreover, the constitutive expression of *lmrC*, *lmrD*, and *lmrR* in the MDR strains indicates that LmrR functions as transcriptional repressor for both *lmrCD* and for its own transcriptions. Transcriptome analysis of the Δ*lmrR* strain showed only the up regulation of the *lmrC* and *lmrD* genes, implying that LmrR is a specific local transcriptional regulator of the expression of *lmrCD*. LmrR binds to two distinct sites in the promoter region of *lmrCD* which

are about 29 base-pairs apart. This separation is longer compared with the other known bacterial repressor DNA binding sites e.g. MarR, a 13 base-pairs separation (92). The *lmrCD* promoter contains two sites for LmrR binding, i.e., site I is located between the -35 and -10 region, whereas site II consists of short imperfect palindrome sequences. Interestingly, *in vitro* site II is not essential for LmrR binding.

Based on homology, LmrR belongs to the PadR family of transcriptional regulators (Pfam PF03551). The palindromic motif of ATGT-8N-ACAT is conserved among the PadR-like regulators, and LmrR binds to a slightly modified but highly homologous motif, i.e. ATGT-10N-ACAT. LmrR also bind to a long stretch of DNA on its own promoter region with no apparent structural features. Interestingly, many of the PadR regulators are involved in the detoxification and enzymatic degradation of phenolic acid, whereas LmrR regulates the expression of an MDR transporter that expels toxic molecules from the cell.

LmrR, like other PadR regulators shares the typical winged-helix turn helix motif in its N-terminal DNA-binding while the C-terminal domain specifies the substrate recognition. LmrR of *L. lactis* and PadR-related proteins of *Staphylococcus*, *Enterococcus*, and *Streptococcus* belong to the subfamily I of PadR protein that are smaller (~110 amino acids long) than the subfamily II proteins to which PadR of *L. lactis* belongs (~ 176 amino acids long) (61). Sequence alignment of LmrR with the members of the PadR (and the more distantly MarR family) indicates the presence of highly conserved amino acyl residues that are located in the hinge region which connects the DNA- and substrate binding domains (9,85), that are critical for DNA binding ability.

The crystal structures of apo LmrR, LmrR bound Hoechst 33342, and LmrR bound daunomycin were solved at 2.0 Å and 2.2 Å, respectively (85). LmrR follows α1-α2-α3-β1-β2-α4 topology with two defined domains. The first domain of the typical winged-helix turn helix DNA binding consists of helices α1, α2, and the DNA recognition helix α3 together with strands β1 and β2 to form the wing. The LmrR protein derived from the rhodamine challenged MDR strain is non functional due to a point mutation of a highly conserved threonine located in the hinge (wing) region of β2. This mutant is unable to bind to the promoter regions of both *lmrCD* and *lmrR* and hence, constitutive transcriptions of these genes take place (2).

Table 1 Transcriptional regulator that control the expression of the MDR pumps in bacteria

Organism	Regulatory protein	Regulatory family	Regulatory function	Regulatory substrates	Regulated gene of MDR pump	Class of MDR pump	References
Eschericia coli	MarA/SoxS/Rob	AraC	Global regulators (activator/repressor)	MarA/SoxS/Rob*	*acrAB* and *tolC*	RND	(88,91,99,100,111)
	MarR	MarR	Repressor of *marA*	DNP, Sa, Md, Pg	*acrAB* and *tolC* via M*arA*	RND	(7,9,92)
	EmrR	MarR	Repressor	Eb, CCCP, DNP, FCCP, Na, Sa, TCS	*emrAB*	MFS	(26,80,189)
	AcrR	TetR	Repressor	Eb, Pf, R6G	*acrAB*	RND	(164)
Staphylococcus aureus	QacR	TetR/CamR	Repressor	Bc, Be, Ch, Cv, Dc, Eb, Mg, Pf, R6G	*qacA/qacB*	MFS	(43,44,152,153)
	MgrA (formerly NorR)	MarR	Global regulators (activator/repressor)	Sf, Cf, Mf, Nf, Ct, Eb, Tc	*norA, norB, norC, tet38*	MFS	(173-175)
Bacillus subtilis	BmrR	MerR	Activator	ABM, ADCP, Ao, DEC, DDPB, R6G, TPP	*bmr*	MFS	(3,178,192)
	BltR	MerR	Activator	unknown	*blt*	MFS	(4)
	Mta	MerR	Global activators	unknown	*bmr* and *blt*	MFS	(13)
Vibrio cholerae	VceR	TetR	Repressor	CCCP, Sa, Dc	*vceCAB*	MFS	(22,186)
	BreR	TetR	Repressor	Ch, Dc, Cc	*breAB*	RND	(27)

Pseudomonas aeruginosa	MexR	MarR	Repressor	unknown	*mexAB-oprM*	RND	(76,163)
Campylobacter jejuni	CmeR	TetR	Repressor	Tch, Gch, Tdc,Ch, Dc,Cc	*cmeABC*	RND	(77,78)
Listeria monocytogenes	LadR	PadR	Repressor	R6G	*mdrL*	MFS	(61)
Lactococcus lactis	LmrR	PadR	Repressor	H33342, Da, Na-Ch	*lmrCD*	ABC	(2)

1). Drugs abbreviation: DNP = 2.4-dinitrophenol, Sa = Salicylate, Md = Menadione, Pg = Plumbagin, Eb = Ethidium bromide, CCCP = carbonyl cyanide *m*-cholorphenylhydrazone, FCCP = carbonyl cyanide *p*-(trifluoro-methoxy), Na = Nalidixic acid, TCS = tetrachlorosalycilamilide, Pf = Proflavine, Bc = Benzalkonium, Be = Berberine, Ch = Chlorhexidine, Cv = Crystal violet, Dc = Dequalinium, R6G = Rhodamine 6G, ABM = 5-(1-adamanthylcarboxyethyl)-3-benzyl-4-methylthiazolium, ADCP = 4-amino-3,6-dimethylbenzo[b]cycloheptano[e]pyridinium, Ao = astrazon orange, DEC = diethyl-2,4'-cyanine, DDPB = 5,6-dichloro-1,3-diethyl-2-(phenylaminovinyl)benzoimidazolium, TPP = tetraphenylphosphonium, Sf = sparfloxacin, Cf = ciprofloxacin, Tc = tetracycline, Dc = deoxycholate, Cdc = Chenodeoxycholate, Tch = taurocholate, Gch = Glycocholate, Ch = cholate, H33342 = Hoechst 33342, Da = daunomycin. **2).** MarA/SoxS/Rob* = MarA and SoxS proteins do not have ligand binding domains

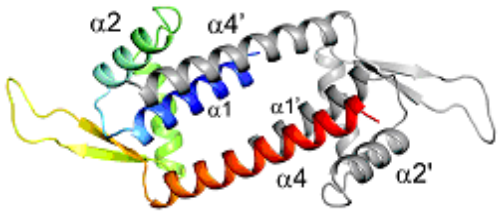


Figure 4. The crystal structure of apo LmrR was solved at 2.0 Å resolution. The N-terminal domain of LmrR is responsible for DNA binding and adopts a winged-HTH motif. The unique architecture of LmrR is marked by the presence of a symmetric central pore formed by the C-terminal domains of the two monomers that is responsible for ligand binding. From reference (85) with permission.

The substrate binding domain of LmrR is made up of a long α4 dimerization helix. The dimerization helix of α4 resembles a protruding arm that intersects with the wHTH domain of the dyad-related subunit with the anti parallel orientation against α1' and interacting with the C-terminal region of α2', and the loop connecting helix α2' and α3'. Interestingly, neither the C-terminal helices of α4 and α4' nor the N-terminal of α1 and α1' interact with each other. A striking structural feature of LmrR is the presence of a large flat-shape central pore for ligand(s) binding. This organization is unique because none of the MarR/PadR related transcriptional regulators possess a central pore at their dimer interface. Moreover, both drug binding sites of BmrR from *B. subtilis* and QacR from *S. aureus* are asymmetric that formed within a single subunit whereas LmrR posses a symmetric binding pocket where both subunits contribute equally to this structure (85). Hoechst 33342 and daunomycin show a common mode of binding: their flat ring systems are wedged in between the W96 and W96' side chains forming aromatic stacking interactions with each of the two indole systems with no hydrogen bonds were observed between drug(s) and LmrR.

A difference in LmrR binding mechanism to the *lmrCD* promoter and its own promoter was demonstrated by Atomic Force Microscopy (AFM) analysis. With a DNA fragment corresponding to the *lmrR* promoter, a severe deformation and supercoiling of the DNA strands occurred upon LmrR binding. This likely to ensures a tight repression of *lmrR* transcription. On the other hand, the binding of LmrR to the *lmrCD* control region introduced DNA bending with the visualization

of two “blobs.” This data suggest the transcription initation by RNA polymerase is possibly being prevented by the binding of two LmrR dimers.

CONCLUDING REMARKS

The emergence of the resistant pathogenic bacterial strains towards a wide range of antibiotics from different classes is progressing in an alarming speed causing a serious threat to public health worldwide. This phenomenon is based on selection for organisms that gained the ability to survive the lethal doses of antibiotics over time. For examples are the *Mycobacterium tuberculosis* (XDR-TB), Methicillin-resistant *Staphylococcus aureus* (MRSA), and Vancomycin-resistant *Enterococcus* (VRE) strains that are highly resistant to different classes of antibiotics available nowadays (1,159). The observed antibiotic resistance in bacteria can be either intrinsic or acquired. Intrinsic resistance is based on the natural mechanisms present in these cells and dependent on the genetic content of the cell. Acquired resistance may relate to different mechanisms such as the occurrence of mutations in the transporter and/or regulatory genes that changed expression and/or selectivity of the MDR transporters, or was obtained by horizontal transfer of the mobile genetic elements that carry genes encoded for resistance to antibiotics (132). Bacteria do not posses specific defense mechanisms to extrude the synthetically introduced antibiotics. Active secretion of drug molecules from the cell results in a multidrug resistance (MDR) phenotype that is often reinforced by the (over)expression of membrane bound MDR transporters. Sequence analysis of a number of bacterial genomes revealed that these transporters appear ubiquitous in nature. One of the known physiological functions of the MDR pumps is to provide an extensive protection against a diverse range of toxic molecules found in their natural environment. The employment and modification of these existing transport systems is subject to regulation by transcriptional regulators that ensure expression of the membrane transporter only when needed in response to the environmental stimuli. Possibly, an excessive production of these proteins is lethal or disadvantageous to the cells.

The study of the mechanism of drug extrusion and recognition by MDR transports is hampered by the difficulty to obtain structural information as these membrane proteins often resist crystallization. On the other hand, the MDR related regulatory proteins are soluble, relatively easy to over express and often can successfully be transformed in high diffracting crystals. These proteins have been showed to contain multiple ligands binding sites that recognized the drugs that are also transported by the MDR transporter that is regulated by these proteins. Thus

they can provide detailed information on the mechanisms of multiple drug recognition. The next stage of analysis is to understand how these drugs affect the ability of these regulators to interact with DNA and how this promotes expression of the designated MDR transporters. Molecular mechanisms causing antibiotic resistance are diverse and often unique for the organism under study. Therefore, a complete, understanding of the mechanisms of regulation and expression of MDR transporter may full future design of novel inhibitors that prevent such resistance mechanisms and thereby increase the life span of currently used antibiotics.

SCOPE OF THE THESIS

The aim of this thesis is to elucidate the molecular basis by which the transcriptional regulator LmrR regulates the expression of the major multidrug resistance ATP-binding cassette transporter LmrCD of *Lactococcus lactis*. Chapter 1 provides an introduction in multidrug resistance transporter and describes the various regulatory mechanism involved in bacterial MDR. Chapter 2 describes the functional and transcriptomic study that identifies LmrCD, a heterodimeric ATP-binding cassette transporter, as the major determinant for both intrinsic and acquired MDR phenotype in *L. lactis*. Cells lacking *lmrCD* genes are hyper sensitive to the presence of several hydrophobic drugs such as Hoechst 33342, daunomycin and ethidium bromide. Conversantly, the overexpression of LmrCD resulted in increased resistances of the cells against the toxic effects of these compounds. Transcriptome analysis on four drug resistance mutant strains of *L. lactis* obtained after a challenge with increasing concentration of daunomycin, ethidium bromide, rhodamine, and cholate revealed the up regulation of *lmrC* and *lmrD* genes. In addition, a common response of these cells is the up regulation of a gene located upstream of *lmrCD* termed *lmrR* (formerly *ydaF*). Sequence analysis suggests that LmrR belongs to the PadR family of transcriptional regulators. A function of LmrR as a transcriptional regulator was confirmed experimentally by its ability to bind to the promoter region of *lmrCD*. The drug resistant phenotype of the selected strains was caused by the constitutive up regulation of *lmrCD* via the premature termination of the *lmrR* gene (Chapter 2) or the expression of a non-functional mutant of LmrR. Chapter 3 describes a further characterization of LmrR. Inactivation of the *lmrR* gene results in a constitutively high expression of the *lmrCD* genes, and does not affect the expression of other genes in *L. lactis* demonstrating that its functions as repressor of *lmrCD* expression only. LmrR binds to its own promoter and to the promoter region of *lmrCD* in two distinct manners. LmrR was found to directly bind drugs suggesting a regulatory

mechanism in which drug binding to LmrR causes the derepression of *lmrCD*. Chapter 4 describes a primer extension analysis showing the presence of multiple transcripts for the *lmrCD* genes and only a single transcript for *lmrR*. Atomic Force Microscopy (AFM) analysis further confirmed major differences in the LmrR binding mode for the *lmrR* and *lmrCD* promoter regions. The binding of LmrR to its own promoter results in a severe DNA deformation and super coiling whereas binding of LmrR to the promoter region of *lmrCD* only causes a distinct DNA bending. Chapter 5 describes a structural analysis of LmrR. The crystal structure of apo LmrR and LmrR bound to either Hoechst 33342 or daunomycin was solved to atomic resolution. The N-terminal domain of LmrR comprises the typical DNA binding winged-helix turn helix motif as found in previously characterized bacterial transcriptional regulators. The LmrR structure also show some unusual features. The two C-terminal regions of the LmrR dimer form a flat-shape hydrophobic central pore that is responsible for drug binding. Hoechst 33342 or daunomycin bind in a similar fashion with their aromatic rings sandwiched in between the indole groups of two dimer-related tryptophan residues. Further functional analysis of site-directed mutants of LmrR suggest an allosteric coupling between the multidrug and DNA binding sites of LmrR that likely plays a role in the induction mechanism. Finally, Chapter 6 summarizes the findings of this thesis and provides an outlook for further research.

REFERENCES

1. Adhikari, R. P., G. C. Scales, K. Kobayashi, J. M. Smith, B. Berger-Bachi, and G. M. Cook. 2004. Vancomycin-induced deletion of the methicillin resistance gene mecA in Staphylococcus aureus. J. Antimicrob. Chemother. 54:360-363.
2. Agustiandari, H., J. Lubelski, van den Berg van Saparoea HB, O. P. Kuipers, and A. J. Driessen. 2008. LmrR is a transcriptional repressor of expression of the multidrug ABC transporter LmrCD in Lactococcus lactis. J. Bacteriol. 190:759-763.
3. Ahmed, M., C. M. Borsch, S. S. Taylor, N. Vazquez-Laslop, and A. A. Neyfakh. 1994. A protein that activates expression of a multidrug efflux transporter upon binding the transporter substrates. J. Biol. Chem. 269:28506-28513.
4. Ahmed, M., L. Lyass, P. N. Markham, S. S. Taylor, N. Vazquez-Laslop, and A. A. Neyfakh. 1995. Two highly similar multidrug transporters of Bacillus subtilis whose expression is differentially regulated. J. Bacteriol. 177:3904-3910.
5. Alekshun, M. N., Y. S. Kim, and S. B. Levy. 2000. Mutational analysis of MarR, the negative regulator of marRAB expression in Escherichia coli, suggests the presence of two regions required for DNA binding. Mol. Microbiol. 35:1394-1404.
6. Alekshun, M. N. and S. B. Levy. 1997. Regulation of chromosomally mediated multiple antibiotic resistance: the mar regulon. Antimicrob. Agents Chemother. 41:2067-2075.

7. Alekshun, M. N. and S. B. Levy. 1999. Alteration of the repressor activity of MarR, the negative regulator of the Escherichia coli marRAB locus, by multiple chemicals in vitro. J. Bacteriol. 181:4669-4672.
8. Alekshun, M. N. and S. B. Levy. 2007. Molecular mechanisms of antibacterial multidrug resistance. Cell 128:1037-1050.
9. Alekshun, M. N., S. B. Levy, T. R. Mealy, B. A. Seaton, and J. F. Head. 2001. The crystal structure of MarR, a regulator of multiple antibiotic resistance, at 2.3 A resolution. Nat. Struct. Biol. 8:710-714.
10. Alguel, Y., C. Meng, W. Teran, T. Krell, J. L. Ramos, M. T. Gallegos, and X. Zhang. 2007. Crystal structures of multidrug binding protein TtgR in complex with antibiotics and plant antimicrobials. J. Mol. Biol. 369:829-840.
11. Alton, N. K. and D. Vapnek. 1979. Nucleotide sequence analysis of the chloramphenicol resistance transposon Tn9. Nature 282:864-869.
12. Ariaratnam, V. and G. P. Georghiou. 1974. Carbamate resistance in Anopheles albimanus. Cross resistance spectrum and stability of resistance. Bull. World Health Organ 51:655-659.
13. Baranova, N. N., A. Danchin, and A. A. Neyfakh. 1999. Mta, a global MerR-type regulator of the Bacillus subtilis multidrug-efflux transporters. Mol. Microbiol. 31:1549-1559.
14. Barbosa, T. M. and S. B. Levy. 2000. Differential expression of over 60 chromosomal genes in Escherichia coli by constitutive expression of MarA. J. Bacteriol. 182:3467-3474.
15. Barbosa, T. M. and S. B. Levy. 2002. Activation of the Escherichia coli nfnB gene by MarA through a highly divergent marbox in a class II promoter. Mol. Microbiol. 45:191-202.
16. Barthelmebs, L., C. Divies, and J. F. Cavin. 2000. Knockout of the p-coumarate decarboxylase gene from Lactobacillus plantarum reveals the existence of two other inducible enzymatic activities involved in phenolic acid metabolism. Appl. Environ. Microbiol. 66:3368-3375.
17. Berens, C., L. Altschmied, and W. Hillen. 1992. The role of the N terminus in Tet repressor for tet operator binding determined by a mutational analysis. J. Biol. Chem. 267:1945-1952.
18. Berens, C., D. Schnappinger, and W. Hillen. 1997. The role of the variable region in Tet repressor for inducibility by tetracycline. J. Biol. Chem. 272:6936-6942.
19. Biedler, J. L. and H. Riehm. 1970. Cellular resistance to actinomycin D in Chinese hamster cells in vitro: cross-resistance, radioautographic, and cytogenetic studies. Cancer Res. 30:1174-1184.
20. Bolhuis, H., D. Molenaar, G. Poelarends, H. W. van Veen, B. Poolman, A. J. Driessen, and W. N. Konings. 1994. Proton motive force-driven and ATP-dependent drug extrusion systems in multidrug-resistant Lactococcus lactis. J. Bacteriol. 176:6957-6964.
21. Bolotin, A., P. Wincker, S. Mauger, O. Jaillon, K. Malarme, J. Weissenbach, S. D. Ehrlich, and A. Sorokin. 2001. The complete genome sequence of the lactic acid bacterium Lactococcus lactis ssp. lactis IL1403. Genome Res. 11:731-753.
22. Borges-Walmsley, M. I., D. Du, K. S. McKeegan, G. J. Sharples, and A. R. Walmsley. 2005. VceR regulates the vceCAB drug efflux pump operon of Vibrio cholerae by alternating between mutually exclusive conformations that bind either drugs or promoter DNA. J. Mol. Biol. 349:387-400.
23. Bosch, I., K. Dunussi-Joannopoulos, R. L. Wu, S. T. Furlong, and J. Croop. 1997. Phosphatidylcholine and phosphatidylethanolamine behave as substrates of the human MDR1 P-glycoprotein. Biochemistry 36:5685-5694.
24. Bouige, P., D. Laurent, L. Piloyan, and E. Dassa. 2002. Phylogenetic and functional classification of ATP-binding cassette (ABC) systems. Curr. Protein Pept. Sci. 3:541-559.
25. Brennan, R. G. and B. W. Matthews. 1989. Structural basis of DNA-protein recognition. Trends Biochem. Sci. 14:286-290.

26. Brooun, A., J. J. Tomashek, and K. Lewis. 1999. Purification and ligand binding of EmrR, a regulator of a multidrug transporter. J. Bacteriol. 181:5131-5133.
27. Cerda-Maira, F. A., C. S. Ringelberg, and R. K. Taylor. 2008. The bile response repressor BreR regulates expression of the Vibrio cholerae breAB efflux system operon. J. Bacteriol. 190:7441-7452.
28. Chopra, I. and M. Roberts. 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol. Mol. Biol. Rev. 65:232-260.
29. Cohen, S. P., W. Yan, and S. B. Levy. 1993. A multidrug resistance regulatory chromosomal locus is widespread among enteric bacteria. J. Infect. Dis. 168:484-488.
30. Dangi, B., P. Pelupessey, R. G. Martin, J. L. Rosner, J. M. Louis, and A. M. Gronenborn. 2001. Structure and dynamics of MarA-DNA complexes: an NMR investigation. J. Mol. Biol. 314:113-127.
31. Dassa, E. and P. Bouige. 2001. The ABC of ABCS: a phylogenetic and functional classification of ABC systems in living organisms. Res. Microbiol. 152:211-229.
32. Dawson, R. J. and K. P. Locher. 2006. Structure of a bacterial multidrug ABC transporter. Nature 443:180-185.
33. De Silva, R. S., G. Kovacikova, W. Lin, R. K. Taylor, K. Skorupski, and F. J. Kull. 2005. Crystal structure of the virulence gene activator AphA from Vibrio cholerae reveals it is a novel member of the winged helix transcription factor superfamily. J. Biol. Chem. 280:13779-13783.
34. Delihas, N. and S. Forst. 2001. MicF: an antisense RNA gene involved in response of Escherichia coli to global stress factors. J. Mol. Biol. 313:1-12.
35. Eckert, B. and C. F. Beck. 1989. Overproduction of transposon Tn10-encoded tetracycline resistance protein results in cell death and loss of membrane potential. J. Bacteriol. 171:3557-3559.
36. Evans, K., L. Adewoye, and K. Poole. 2001. MexR repressor of the mexAB-oprM multidrug efflux operon of Pseudomonas aeruginosa: identification of MexR binding sites in the mexA-mexR intergenic region. J. Bacteriol. 183:807-812.
37. Eytan, G. D., M. J. Borgnia, R. Regev, and Y. G. Assaraf. 1994. Transport of polypeptide ionophores into proteoliposomes reconstituted with rat liver P-glycoprotein. J. Biol. Chem. 269:26058-26065.
38. Fromknecht, K., P. D. Vogel, and J. G. Wise. 2003. Combinatorial redesign of the DNA binding specificity of a prokaryotic helix-turn-helix repressor. J. Bacteriol. 185:475-481.
39. Gajiwala, K. S. and S. K. Burley. 2000. Winged helix proteins. Curr. Opin. Struct. Biol. 10:110-116.
40. Gasson, M. J., Y. Kitamura, W. R. McLauchlan, A. Narbad, A. J. Parr, E. L. Parsons, J. Payne, M. J. Rhodes, and N. J. Walton. 1998. Metabolism of ferulic acid to vanillin. A bacterial gene of the enoyl-SCoA hydratase/isomerase superfamily encodes an enzyme for the hydration and cleavage of a hydroxycinnamic acid SCoA thioester. J. Biol. Chem. 273:4163-4170.
41. Georghiou, G. P. 1965. Genetic studies on insecticide resistance. Adv. Pest. Control Res. 6:171-230.
42. Gillette, W. K., R. G. Martin, and J. L. Rosner. 2000. Probing the Escherichia coli transcriptional activator MarA using alanine-scanning mutagenesis: residues important for DNA binding and activation. J. Mol. Biol. 299:1245-1255.
43. Grkovic, S., M. H. Brown, N. J. Roberts, I. T. Paulsen, and R. A. Skurray. 1998. QacR is a repressor protein that regulates expression of the Staphylococcus aureus multidrug efflux pump QacA. J. Biol. Chem. 273:18665-18673.

44. Grkovic, S., M. H. Brown, M. A. Schumacher, R. G. Brennan, and R. A. Skurray. 2001. The staphylococcal QacR multidrug regulator binds a correctly spaced operator as a pair of dimers. J. Bacteriol. 183:7102-7109.
45. Grkovic, S., M. H. Brown, and R. A. Skurray. 2001. Transcriptional regulation of multidrug efflux pumps in bacteria. Semin. Cell Dev. Biol. 12:225-237.
46. Grkovic, S., M. H. Brown, and R. A. Skurray. 2002. Regulation of bacterial drug export systems. Microbiol. Mol. Biol. Rev. 66:671-701, table.
47. Grkovic, S., K. M. Hardie, M. H. Brown, and R. A. Skurray. 2003. Interactions of the QacR multidrug-binding protein with structurally diverse ligands: implications for the evolution of the binding pocket. Biochemistry 42:15226-15236.
48. Gury, J., L. Barthelmebs, N. P. Tran, C. Divies, and J. F. Cavin. 2004. Cloning, deletion, and characterization of PadR, the transcriptional repressor of the phenolic acid decarboxylase-encoding padA gene of Lactobacillus plantarum. Appl. Environ. Microbiol. 70:2146-2153.
49. Harbottle, H., S. Thakur, S. Zhao, and D. G. White. 2006. Genetics of antimicrobial resistance. Anim Biotechnol. 17:111-124.
50. Harrison, S. C. 1991. A structural taxonomy of DNA-binding domains. Nature 353:715-719.
51. Harrison, S. C. and A. K. Aggarwal. 1990. DNA recognition by proteins with the helix-turn-helix motif. Annu. Rev. Biochem. 59:933-969.
52. Hecht, B., G. Muller, and W. Hillen. 1993. Noninducible Tet repressor mutations map from the operator binding motif to the C terminus. J. Bacteriol. 175:1206-1210.
53. Helbl, V., C. Berens, and W. Hillen. 1995. Proximity probing of Tet repressor to tet operator by dimethylsulfate reveals protected and accessible functions for each recognized base-pair in the major groove. J. Mol. Biol. 245:538-548.
54. Heldwein, E. E. and R. G. Brennan. 2001. Crystal structure of the transcription activator BmrR bound to DNA and a drug. Nature 409:378-382.
55. Higgins, C. F. 1992. ABC transporters: from microorganisms to man. Annu. Rev. Cell Biol. 8:67-113.
56. Hillen, W. and C. Berens. 1994. Mechanisms underlying expression of Tn10 encoded tetracycline resistance. Annu. Rev. Microbiol. 48:345-369.
57. Hinrichs, W., C. Kisker, M. Duvel, A. Muller, K. Tovar, W. Hillen, and W. Saenger. 1994. Structure of the Tet repressor-tetracycline complex and regulation of antibiotic resistance. Science 264:418-420.
58. Holmes, D. J., J. L. Caso, and C. J. Thompson. 1993. Autogenous transcriptional activation of a thiostrepton-induced gene in Streptomyces lividans. EMBO J. 12:3183-3191.
59. Holtje, J. V. 1998. Growth of the stress-bearing and shape-maintaining murein sacculus of Escherichia coli. Microbiol. Mol. Biol. Rev. 62:181-203.
60. Holtz, T. H. and J. P. Cegielski. 2007. Origin of the term XDR-TB. Eur. Respir. J. 30:396.
61. Huillet, E., P. Velge, T. Vallaeys, and P. Pardon. 2006. LadR, a new PadR-related transcriptional regulator from Listeria monocytogenes, negatively regulates the expression of the multidrug efflux pump MdrL. FEMS Microbiol. Lett. 254:87-94.
62. Ishida, H., H. Fuziwara, Y. Kaibori, T. Horiuchi, K. Sato, and Y. Osada. 1995. Cloning of multidrug resistance gene pqrA from Proteus vulgaris. Antimicrob. Agents Chemother. 39:453-457.
63. Jain, A. and R. Mondal. 2008. Extensively drug-resistant tuberculosis: current challenges and threats. FEMS Immunol. Med. Microbiol. 53:145-150.
64. Jair, K. W., W. P. Fawcett, N. Fujita, A. Ishihama, and R. E. Wolf, Jr. 1996. Ambidextrous transcriptional activation by SoxS: requirement for the C-terminal domain of the RNA polymerase alpha subunit in a subset of Escherichia coli superoxide-inducible genes. Mol. Microbiol. 19:307-317.

65. Jair, K. W., X. Yu, K. Skarstad, B. Thony, N. Fujita, A. Ishihama, and R. E. Wolf, Jr. 1996. Transcriptional activation of promoters of the superoxide and multiple antibiotic resistance regulons by Rob, a binding protein of the Escherichia coli origin of chromosomal replication. J. Bacteriol. 178:2507-2513.
66. Jellen-Ritter, A. S. and W. V. Kern. 2001. Enhanced expression of the multidrug efflux pumps AcrAB and AcrEF associated with insertion element transposition in Escherichia coli mutants Selected with a fluoroquinolone. Antimicrob. Agents Chemother. 45:1467-1472.
67. Johnson, I. S., J. G. Armstrong, M. Gorman, and J. P. Burnett, Jr. 1963. The Vincalkaloids: A new class of oncolytic agents. Cancer Res. 23:1390-1427.
68. Kazama, H., H. Hamashima, M. Sasatsu, and T. Arai. 1998. Distribution of the antiseptic-resistance gene qacE delta 1 in gram-positive bacteria. FEMS Microbiol. Lett. 165:295-299.
69. Kleinschmidt, C., K. Tovar, and W. Hillen. 1991. Computer simulations and experimental studies of gel mobility patterns for weak and strong non-cooperative protein binding to two targets on the same DNA: application to binding of tet repressor variants to multiple and single tet operator sites. Nucleic Acids Res. 19:1021-1028.
70. Knol, J., L. Veenhoff, W. J. Liang, P. J. Henderson, G. Leblanc, and B. Poolman. 1996. Unidirectional reconstitution into detergent-destabilized liposomes of the purified lactose transport system of Streptococcus thermophilus. J. Biol. Chem. 271:15358-15366.
71. Kovacikova, G., W. Lin, and K. Skorupski. 2003. The virulence activator AphA links quorum sensing to pathogenesis and physiology in Vibrio cholerae by repressing the expression of a penicillin amidase gene on the small chromosome. J. Bacteriol. 185:4825-4836.
72. Kovacikova, G., W. Lin, and K. Skorupski. 2004. Vibrio cholerae AphA uses a novel mechanism for virulence gene activation that involves interaction with the LysR-type regulator AphB at the tcpPH promoter. Mol. Microbiol. 53:129-142.
73. Kuroda, M., T. Ohta, I. Uchiyama, T. Baba, H. Yuzawa, I. Kobayashi, L. Cui, A. Oguchi, K. Aoki, Y. Nagai, J. Lian, T. Ito, M. Kanamori, H. Matsumaru, A. Maruyama, H. Murakami, A. Hosoyama, Y. Mizutani-Ui, N. K. Takahashi, T. Sawano, R. Inoue, C. Kaito, K. Sekimizu, H. Hirakawa, S. Kuhara, S. Goto, J. Yabuzaki, M. Kanehisa, A. Yamashita, K. Oshima, K. Furuya, C. Yoshino, T. Shiba, M. Hattori, N. Ogasawara, H. Hayashi, and K. Hiramatsu. 2001. Whole genome sequencing of meticillin-resistant Staphylococcus aureus. Lancet 357:1225-1240.
74. Lee, E. H., E. Collatz, I. Podglajen, and L. Gutmann. 1996. A rob-like gene of Enterobacter cloacae affecting porin synthesis and susceptibility to multiple antibiotics. Antimicrob. Agents Chemother. 40:2029-2033.
75. Levy, S. B. and B. Marshall. 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nat. Med. 10:S122-S129.
76. Lim, D., K. Poole, and N. C. Strynadka. 2002. Crystal structure of the MexR repressor of the mexRAB-oprM multidrug efflux operon of Pseudomonas aeruginosa. J. Biol. Chem. 277:29253-29259.
77. Lin, J., M. Akiba, O. Sahin, and Q. Zhang. 2005. CmeR functions as a transcriptional repressor for the multidrug efflux pump CmeABC in Campylobacter jejuni. Antimicrob. Agents Chemother. 49:1067-1075.
78. Lin, J., C. Cagliero, B. Guo, Y. W. Barton, M. C. Maurel, S. Payot, and Q. Zhang. 2005. Bile salts modulate expression of the CmeABC multidrug efflux pump in Campylobacter jejuni. J. Bacteriol. 187:7417-7424.
79. Littlejohn, T. G., I. T. Paulsen, M. T. Gillespie, J. M. Tennent, M. Midgley, I. G. Jones, A. S. Purewal, and R. A. Skurray. 1992. Substrate specificity and energetics of antiseptic and disinfectant resistance in Staphylococcus aureus. FEMS Microbiol. Lett. 74:259-265.

80. Lomovskaya, O., K. Lewis, and A. Matin. 1995. EmrR is a negative regulator of the Escherichia coli multidrug resistance pump EmrAB. J. Bacteriol. 177:2328-2334.
81. Lubelski, J., J. A. de, M. R. van, H. Agustiandari, O. P. Kuipers, J. Kok, and A. J. Driessen. 2006. LmrCD is a major multidrug resistance transporter in Lactococcus lactis. Mol. Microbiol. 61:771-781.
82. Lubelski, J., P. Mazurkiewicz, M. R. van, W. N. Konings, and A. J. Driessen. 2004. ydaG and ydbA of Lactococcus lactis encode a heterodimeric ATP-binding cassette-type multidrug transporter. J. Biol. Chem. 279:34449-34455.
83. Ma, D., M. Alberti, C. Lynch, H. Nikaido, and J. E. Hearst. 1996. The local repressor AcrR plays a modulating role in the regulation of acrAB genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112.
84. Ma, D., D. N. Cook, M. Alberti, N. G. Pon, H. Nikaido, and J. E. Hearst. 1995. Genes acrA and acrB encode a stress-induced efflux system of Escherichia coli. Mol. Microbiol. 16:45-55.
85. Madoori, P. K., H. Agustiandari, A. J. Driessen, and A. M. Thunnissen. 2008. Structure of the transcriptional regulator LmrR and its mechanism of multidrug recognition. EMBO J.
86. Margolles, A., M. Putman, H. W. van Veen, and W. N. Konings. 1999. The purified and functionally reconstituted multidrug transporter LmrA of Lactococcus lactis mediates the transbilayer movement of specific fluorescent phospholipids. Biochemistry 38:16298-16306.
87. Markham, P. N., M. Ahmed, and A. A. Neyfakh. 1996. The drug-binding activity of the multidrug-responding transcriptional regulator BmrR resides in its C-terminal domain. J. Bacteriol. 178:1473-1475.
88. Martin, R. G., E. S. Bartlett, J. L. Rosner, and M. E. Wall. 2008. Activation of the Escherichia coli marA/soxS/rob regulon in response to transcriptional activator concentration. J. Mol. Biol. 380:278-284.
89. Martin, R. G., W. K. Gillette, N. I. Martin, and J. L. Rosner. 2002. Complex formation between activator and RNA polymerase as the basis for transcriptional activation by MarA and SoxS in Escherichia coli. Mol. Microbiol. 43:355-370.
90. Martin, R. G., W. K. Gillette, S. Rhee, and J. L. Rosner. 1999. Structural requirements for marbox function in transcriptional activation of mar/sox/rob regulon promoters in Escherichia coli: sequence, orientation and spatial relationship to the core promoter. Mol. Microbiol. 34:431-441.
91. Martin, R. G., K. W. Jair, R. E. Wolf, Jr., and J. L. Rosner. 1996. Autoactivation of the marRAB multiple antibiotic resistance operon by the MarA transcriptional activator in Escherichia coli. J. Bacteriol. 178:2216-2223.
92. Martin, R. G. and J. L. Rosner. 1995. Binding of purified multiple antibiotic-resistance repressor protein (MarR) to mar operator sequences. Proc. Natl. Acad. Sci. U. S. A 92:5456-5460.
93. Martin, R. G. and J. L. Rosner. 1997. Fis, an accessorial factor for transcriptional activation of the mar (multiple antibiotic resistance) promoter of Escherichia coli in the presence of the activator MarA, SoxS, or Rob. J. Bacteriol. 179:7410-7419.
94. Martin, R. G. and J. L. Rosner. 2001. The AraC transcriptional activators. Curr. Opin. Microbiol. 4:132-137.
95. Martin, R. G. and J. L. Rosner. 2002. Genomics of the marA/soxS/rob regulon of Escherichia coli: identification of directly activated promoters by application of molecular genetics and informatics to microarray data. Mol. Microbiol. 44:1611-1624.
96. Mazzariol, A., Y. Tokue, T. M. Kanegawa, G. Cornaglia, and H. Nikaido. 2000. High-level fluoroquinolone-resistant clinical isolates of Escherichia coli overproduce multidrug efflux protein AcrA. Antimicrob. Agents Chemother. 44:3441-3443.

97. McDermott, P. F., D. G. White, I. Podglajen, M. N. Alekshun, and S. B. Levy. 1998. Multidrug resistance following expression of the Escherichia coli marA gene in Mycobacterium smegmatis. J. Bacteriol. 180:2995-2998.
98. Meier, I., L. V. Wray, and W. Hillen. 1988. Differential regulation of the Tn10-encoded tetracycline resistance genes tetA and tetR by the tandem tet operators O1 and O2. EMBO J. 7:567-572.
99. Michan, C., M. Manchado, and C. Pueyo. 2002. SoxRS down-regulation of rob transcription. J. Bacteriol. 184:4733-4738.
100. Miller, P. F., L. F. Gambino, M. C. Sulavik, and S. J. Gracheck. 1994. Genetic relationship between soxRS and mar loci in promoting multiple antibiotic resistance in Escherichia coli. Antimicrob. Agents Chemother. 38:1773-1779.
101. Miller, P. F. and M. C. Sulavik. 1996. Overlaps and parallels in the regulation of intrinsic multiple-antibiotic resistance in Escherichia coli. Mol. Microbiol. 21:441-448.
102. Mitchell, B. A., M. H. Brown, and R. A. Skurray. 1998. QacA multidrug efflux pump from Staphylococcus aureus: comparative analysis of resistance to diamidines, biguanidines, and guanylhydrazones. Antimicrob. Agents Chemother. 42:475-477.
103. Mitchell, B. A., I. T. Paulsen, M. H. Brown, and R. A. Skurray. 1999. Bioenergetics of the staphylococcal multidrug export protein QacA. Identification of distinct binding sites for monovalent and divalent cations. J. Biol. Chem. 274:3541-3548.
104. Muller, G., B. Hecht, V. Helbl, W. Hinrichs, W. Saenger, and W. Hillen. 1995. Characterization of non-inducible Tet repressor mutants suggests conformational changes necessary for induction. Nat. Struct. Biol. 2:693-703.
105. Neu, H. C. 1992. The crisis in antibiotic resistance. Science 257:1064-1073.
106. Ng, E. Y., M. Trucksis, and D. C. Hooper. 1994. Quinolone resistance mediated by norA: physiologic characterization and relationship to flqB, a quinolone resistance locus on the Staphylococcus aureus chromosome. Antimicrob. Agents Chemother. 38:1345-1355.
107. Nikaido, H. 1994. Prevention of drug access to bacterial targets: permeability barriers and active efflux. Science 264:382-388.
108. Nikaido, H. and D. G. Thanassi. 1993. Penetration of lipophilic agents with multiple protonation sites into bacterial cells: tetracyclines and fluoroquinolones as examples. Antimicrob. Agents Chemother. 37:1393-1399.
109. Nikaido, H. and H. I. Zgurskaya. 2001. AcrAB and related multidrug efflux pumps of Escherichia coli. J. Mol. Microbiol. Biotechnol. 3:215-218.
110. Notka, F., H. J. Linde, A. Dankesreiter, H. H. Niller, and N. Lehn. 2002. A C-terminal 18 amino acid deletion in MarR in a clinical isolate of Escherichia coli reduces MarR binding properties and increases the MIC of ciprofloxacin. J. Antimicrob. Chemother. 49:41-47.
111. Nunoshiba, T., E. Hidalgo, Z. Li, and B. Demple. 1993. Negative autoregulation by the Escherichia coli SoxS protein: a dampening mechanism for the soxRS redox stress response. J. Bacteriol. 175:7492-7494.
112. Nunoshiba, T., E. Hidalgo, C. F. mabile Cuevas, and B. Demple. 1992. Two-stage control of an oxidative stress regulon: the Escherichia coli SoxR protein triggers redox-inducible expression of the soxS regulatory gene. J. Bacteriol. 174:6054-6060.
113. Oethinger, M., I. Podglajen, W. V. Kern, and S. B. Levy. 1998. Overexpression of the marA or soxS regulatory gene in clinical topoisomerase mutants of Escherichia coli. Antimicrob. Agents Chemother. 42:2089-2094.
114. Okusu, H., D. Ma, and H. Nikaido. 1996. AcrAB efflux pump plays a major role in the antibiotic resistance phenotype of Escherichia coli multiple-antibiotic-resistance (Mar) mutants. J. Bacteriol. 178:306-308.

115. Orth, P., F. Cordes, D. Schnappinger, W. Hillen, W. Saenger, and W. Hinrichs. 1998. Conformational changes of the Tet repressor induced by tetracycline trapping. J. Mol. Biol. 279:439-447.
116. Orth, P., W. Saenger, and W. Hinrichs. 1999. Tetracycline-chelated Mg2+ ion initiates helix unwinding in Tet repressor induction. Biochemistry 38:191-198.
117. Orth, P., D. Schnappinger, W. Hillen, W. Saenger, and W. Hinrichs. 2000. Structural basis of gene regulation by the tetracycline inducible Tet repressor-operator system. Nat. Struct. Biol. 7:215-219.
118. Orth, P., D. Schnappinger, P. E. Sum, G. A. Ellestad, W. Hillen, W. Saenger, and W. Hinrichs. 1999. Crystal structure of the tet repressor in complex with a novel tetracycline, 9-(N,N-dimethylglycylamido)- 6-demethyl-6-deoxy-tetracycline. J. Mol. Biol. 285:455-461.
119. Overhage, J., H. Priefert, and A. Steinbuchel. 1999. Biochemical and genetic analyses of ferulic acid catabolism in Pseudomonas sp. Strain HR199. Appl. Environ. Microbiol. 65:4837-4847.
120. Pabo, C. O. and R. T. Sauer. 1992. Transcription factors: structural families and principles of DNA recognition. Annu. Rev. Biochem. 61:1053-1095.
121. Palumbi, S. R. 2001. Humans as the world's greatest evolutionary force. Science 293:1786-1790.
122. Paulsen, I. T., M. H. Brown, T. G. Littlejohn, B. A. Mitchell, and R. A. Skurray. 1996. Multidrug resistance proteins QacA and QacB from Staphylococcus aureus: membrane topology and identification of residues involved in substrate specificity. Proc. Natl. Acad. Sci. U. S. A 93:3630-3635.
123. Paulsen, I. T., M. H. Brown, and R. A. Skurray. 1996. Proton-dependent multidrug efflux systems. Microbiol. Rev. 60:575-608.
124. Paulsen, I. T., M. H. Brown, and R. A. Skurray. 1998. Characterization of the earliest known Staphylococcus aureus plasmid encoding a multidrug efflux system. J. Bacteriol. 180:3477-3479.
125. Paulsen, I. T., L. Nguyen, M. K. Sliwinski, R. Rabus, and M. H. Saier, Jr. 2000. Microbial genome analyses: comparative transport capabilities in eighteen prokaryotes. J. Mol. Biol. 301:75-100.
126. Paulsen, I. T., M. K. Sliwinski, and M. H. Saier, Jr. 1998. Microbial genome analyses: global comparisons of transport capabilities based on phylogenies, bioenergetics and substrate specificities. J. Mol. Biol. 277:573-592.
127. Perez-Rueda, E. and J. Collado-Vides. 2001. Common history at the origin of the position-function correlation in transcriptional regulators in archaea and bacteria. J. Mol. Evol. 53:172-179.
128. Pomposiello, P. J., M. H. Bennik, and B. Demple. 2001. Genome-wide transcriptional profiling of the Escherichia coli responses to superoxide stress and sodium salicylate. J. Bacteriol. 183:3890-3902.
129. Poole, K. 1994. Bacterial multidrug resistance--emphasis on efflux mechanisms and Pseudomonas aeruginosa. J. Antimicrob. Chemother. 34:453-456.
130. Poole, K., D. E. Heinrichs, and S. Neshat. 1993. Cloning and sequence analysis of an EnvCD homologue in Pseudomonas aeruginosa: regulation by iron and possible involvement in the secretion of the siderophore pyoverdine. Mol. Microbiol. 10:529-544.
131. Poole, K., K. Tetro, Q. Zhao, S. Neshat, D. E. Heinrichs, and N. Bianco. 1996. Expression of the multidrug resistance operon mexA-mexB-oprM in Pseudomonas aeruginosa: mexR encodes a regulator of operon expression. Antimicrob. Agents Chemother. 40:2021-2028.
132. Putman, M., H. W. van Veen, and W. N. Konings. 2000. Molecular properties of bacterial multidrug transporters. Microbiol. Mol. Biol. Rev. 64:672-693.

133. Putman, M., H. W. van Veen, B. Poolman, and W. N. Konings. 1999. Restrictive use of detergents in the functional reconstitution of the secondary multidrug transporter LmrP. Biochemistry 38:1002-1008.
134. Rahmati, S., S. Yang, A. L. Davidson, and E. L. Zechiedrich. 2002. Control of the AcrAB multidrug efflux pump by quorum-sensing regulator SdiA. Mol. Microbiol. 43:677-685.
135. Ramos, J. L., M. Martinez-Bueno, A. J. Molina-Henares, W. Teran, K. Watanabe, X. Zhang, M. T. Gallegos, R. Brennan, and R. Tobes. 2005. The TetR family of transcriptional repressors. Microbiol. Mol. Biol. Rev. 69:326-356.
136. Rand, J. D., S. G. Danby, D. L. Greenway, and R. R. England. 2002. Increased expression of the multidrug efflux genes acrAB occurs during slow growth of Escherichia coli. FEMS Microbiol. Lett. 207:91-95.
137. Rhee, S., R. G. Martin, J. L. Rosner, and D. R. Davies. 1998. A novel DNA-binding motif in MarA: the first structure for an AraC family transcriptional activator. Proc. Natl. Acad. Sci. U. S. A 95:10413-10418.
138. Rosner, J. L., B. Dangi, A. M. Gronenborn, and R. G. Martin. 2002. Posttranscriptional activation of the transcriptional activator Rob by dipyridyl in Escherichia coli. J. Bacteriol. 184:1407-1416.
139. Rouch, D. A., D. S. Cram, D. DiBerardino, T. G. Littlejohn, and R. A. Skurray. 1990. Efflux-mediated antiseptic resistance gene qacA from Staphylococcus aureus: common ancestry with tetracycline- and sugar-transport proteins. Mol. Microbiol. 4:2051-2062.
140. Rousseau, P., E. Gueguen, G. Duval-Valentin, and M. Chandler. 2004. The helix-turn-helix motif of bacterial insertion sequence IS911 transposase is required for DNA binding. Nucleic Acids Res. 32:1335-1344.
141. Saier, M. H., Jr. and I. T. Paulsen. 2001. Phylogeny of multidrug transporters. Semin. Cell Dev. Biol. 12:205-213.
142. Saito, K., H. Yoneyama, and T. Nakae. 1999. nalB-type mutations causing the overexpression of the MexAB-OprM efflux pump are located in the mexR gene of the Pseudomonas aeruginosa chromosome. FEMS Microbiol. Lett. 179:67-72.
143. Sawada, I., H. Maseda, T. Nakae, H. Uchiyama, and N. Nomura. 2004. A quorum-sensing autoinducer enhances the mexAB-oprM efflux-pump expression without the MexR-mediated regulation in Pseudomonas aeruginosa. Microbiol. Immunol. 48:435-439.
144. Schnappinger, D. and W. Hillen. 1996. Tetracyclines: antibiotic action, uptake, and resistance mechanisms. Arch. Microbiol. 165:359-369.
145. Schnappinger, D., P. Schubert, C. Berens, K. Pfleiderer, and W. Hillen. 1999. Solvent-exposed residues in the Tet repressor (TetR) four-helix bundle contribute to subunit recognition and dimer stability. J. Biol. Chem. 274:6405-6410.
146. Schnappinger, D., P. Schubert, K. Pfleiderer, and W. Hillen. 1998. Determinants of protein-protein recognition by four helix bundles: changing the dimerization specificity of Tet repressor. EMBO J. 17:535-543.
147. Schneiders, T. and S. B. Levy. 2006. MarA-mediated transcriptional repression of the rob promoter. J. Biol. Chem. 281:10049-10055.
148. Scholz, O., M. Kintrup, M. Reich, and W. Hillen. 2001. Mechanism of Tet repressor induction by tetracyclines: length compensates for sequence in the alpha8-alpha9 loop. J. Mol. Biol. 310:979-986.
149. Scholz, O., P. Schubert, M. Kintrup, and W. Hillen. 2000. Tet repressor induction without Mg2+. Biochemistry 39:10914-10920.
150. Schubert, P., D. Schnappinger, K. Pfleiderer, and W. Hillen. 2001. Identification of a stability determinant on the edge of the Tet repressor four-helix bundle dimerization motif. Biochemistry 40:3257-3263.

151. Schumacher, M. A., M. C. Miller, and R. G. Brennan. 2004. Structural mechanism of the simultaneous binding of two drugs to a multidrug-binding protein. EMBO J. 23:2923-2930.
152. Schumacher, M. A., M. C. Miller, S. Grkovic, M. H. Brown, R. A. Skurray, and R. G. Brennan. 2001. Structural mechanisms of QacR induction and multidrug recognition. Science 294:2158-2163.
153. Schumacher, M. A., M. C. Miller, S. Grkovic, M. H. Brown, R. A. Skurray, and R. G. Brennan. 2002. Structural basis for cooperative DNA binding by two dimers of the multidrug-binding protein QacR. EMBO J. 21:1210-1218.
154. Segura, A., P. V. Bunz, D. A. D'Argenio, and L. N. Ornston. 1999. Genetic analysis of a chromosomal region containing vanA and vanB, genes required for conversion of either ferulate or vanillate to protocatechuate in Acinetobacter. J. Bacteriol. 181:3494-3504.
155. Seoane, A. S. and S. B. Levy. 1995. Characterization of MarR, the repressor of the multiple antibiotic resistance (mar) operon in Escherichia coli. J. Bacteriol. 177:3414-3419.
156. Sharom, F. J., G. Diodato, X. Yu, and K. J. Ashbourne. 1995. Interaction of the P-glycoprotein multidrug transporter with peptides and ionophores. J. Biol. Chem. 270:10334-10341.
157. Sheps, J. A. and V. Ling. 2007. Preface: the concept and consequences of multidrug resistance. Pflugers Arch. 453:545-553.
158. Smith, L. D. and K. P. Bertrand. 1988. Mutations in the Tn10 tet repressor that interfere with induction. Location of the tetracycline-binding domain. J. Mol. Biol. 203:949-959.
159. Sood, S., M. Malhotra, B. K. Das, and A. Kapil. 2008. Enterococcal infections & antimicrobial resistance. Indian J. Med. Res. 128:111-121.
160. Speer, B. S., N. B. Shoemaker, and A. A. Salyers. 1992. Bacterial resistance to tetracycline: mechanisms, transfer, and clinical significance. Clin. Microbiol. Rev. 5:387-399.
161. Spott, S., F. Dong, B. Kisters-Woike, and B. Muller-Hill. 2000. Dimerisation mutants of Lac repressor. II. A single amino acid substitution, D278L, changes the specificity of dimerisation. J. Mol. Biol. 296:673-684.
162. Spratt, B. G. 1994. Resistance to antibiotics mediated by target alterations. Science 264:388-393.
163. Srikumar, R., C. J. Paul, and K. Poole. 2000. Influence of mutations in the mexR repressor gene on expression of the MexA-MexB-oprM multidrug efflux system of Pseudomonas aeruginosa. J. Bacteriol. 182:1410-1414.
164. Su, C. C., D. J. Rutherford, and E. W. Yu. 2007. Characterization of the multidrug efflux regulator AcrR from Escherichia coli. Biochem. Biophys. Res. Commun. 361:85-90.
165. Tanaka, T., T. Horii, K. Shibayama, K. Sato, S. Ohsuka, Y. Arakawa, K. Yamaki, K. Takagi, and M. Ohta. 1997. RobA-induced multiple antibiotic resistance largely depends on the activation of the AcrAB efflux. Microbiol. Immunol. 41:697-702.
166. Tennent, J. M., B. R. Lyon, M. Midgley, I. G. Jones, A. S. Purewal, and R. A. Skurray. 1989. Physical and biochemical characterization of the qacA gene encoding antiseptic and disinfectant resistance in Staphylococcus aureus. J. Gen. Microbiol. 135:1-10.
167. Tennent, J. M., J. W. May, and R. A. Skurray. 1984. Multiple antibiotic resistance in Staphylococcus aureus and Staphylococcus epidermidis: plasmids in strains associated with nosocomial infection. Pathology 16:250-255.
168. Thanassi, D. G., L. W. Cheng, and H. Nikaido. 1997. Active efflux of bile salts by Escherichia coli. J. Bacteriol. 179:2512-2518.
169. Tiebel, B., K. Garke, and W. Hillen. 2000. Observing conformational and activity changes of tet repressor in vivo. Nat. Struct. Biol. 7:479-481.

170. Tiebel, B., N. Radzwill, L. M. ung-Hilbrich, V. Helbl, H. J. Steinhoff, and W. Hillen. 1999. Domain motions accompanying Tet repressor induction defined by changes of interspin distances at selectively labeled sites. J. Mol. Biol. 290:229-240.
171. Tiebel, B., L. M. ung-Hilbrich, D. Schnappinger, and W. Hillen. 1998. Conformational changes necessary for gene regulation by Tet repressor assayed by reversible disulfide bond formation. EMBO J. 17:5112-5119.
172. Travers, A., R. Schneider, and G. Muskhelishvili. 2001. DNA supercoiling and transcription in Escherichia coli: The FIS connection. Biochimie 83:213-217.
173. Truong-Bolduc, Q. C., P. M. Dunman, J. Strahilevitz, S. J. Projan, and D. C. Hooper. 2005. MgrA is a multiple regulator of two new efflux pumps in Staphylococcus aureus. J. Bacteriol. 187:2395-2405.
174. Truong-Bolduc, Q. C., J. Strahilevitz, and D. C. Hooper. 2006. NorC, a new efflux pump regulated by MgrA of Staphylococcus aureus. Antimicrob. Agents Chemother. 50:1104-1107.
175. Truong-Bolduc, Q. C., X. Zhang, and D. C. Hooper. 2003. Characterization of NorR protein, a multifunctional regulator of norA expression in Staphylococcus aureus. J. Bacteriol. 185:3127-3138.
176. Ubukata, K., N. Itoh-Yamashita, and M. Konno. 1989. Cloning and expression of the norA gene for fluoroquinolone resistance in Staphylococcus aureus. Antimicrob. Agents Chemother. 33:1535-1539.
177. van, H. A., A. J. Smith, H. Sprong, I. Fritzsche, A. H. Schinkel, P. Borst, and M. G. van. 1996. MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine. Cell 87:507-517.
178. Vazquez-Laslop, N., P. N. Markham, and A. A. Neyfakh. 1999. Mechanism of ligand recognition by BmrR, the multidrug-responding transcriptional regulator: mutational analysis of the ligand-binding site. Biochemistry 38:16925-16931.
179. Vergani, B., M. Kintrup, W. Hillen, H. Lami, E. Piemont, E. Bombarda, P. Alberti, S. M. Doglia, and M. Chabbert. 2000. Backbone dynamics of Tet repressor alpha8intersectionalpha9 loop. Biochemistry 39:2759-2768.
180. von, H. G. 1991. Proline kinks in transmembrane alpha-helices. J. Mol. Biol. 218:499-503.
181. Wang, H., J. L. Dzink-Fox, M. Chen, and S. B. Levy. 2001. Genetic characterization of highly fluoroquinolone-resistant clinical Escherichia coli strains from China: role of acrR mutations. Antimicrob. Agents Chemother. 45:1515-1521.
182. Webber, M. A. and L. J. Piddock. 2001. Absence of mutations in marRAB or soxRS in acrB-overexpressing fluoroquinolone-resistant clinical and veterinary isolates of Escherichia coli. Antimicrob. Agents Chemother. 45:1550-1552.
183. Wegmann, U., M. O'Connell-Motherway, A. Zomer, G. Buist, C. Shearman, C. Canchaya, M. Ventura, A. Goesmann, M. J. Gasson, O. P. Kuipers, D. van Sinderen, and J. Kok. 2007. Complete genome sequence of the prototype lactic acid bacterium *Lactococcus lactis* subsp. *cremoris* MG1363. J. Bacteriol. 189:3256-3270.
184. White, D. G., J. D. Goldman, B. Demple, and S. B. Levy. 1997. Role of the acrAB locus in organic solvent tolerance mediated by expression of marA, soxS, or robA in Escherichia coli. J. Bacteriol. 179:6122-6126.
185. Woodcock, D. M., M. E. Linsenmeyer, G. Chojnowski, A. B. Kriegler, V. Nink, L. K. Webster, and W. H. Sawyer. 1992. Reversal of multidrug resistance by surfactants. Br. J. Cancer 66:62-68.
186. Woolley, R. C., G. Vediyappan, M. Anderson, M. Lackey, B. Ramasubramanian, B. Jiangping, T. Borisova, J. A. Colmer, A. N. Hamood, C. S. McVay, and J. A. Fralick. 2005.

Characterization of the Vibrio cholerae vceCAB multiple-drug resistance efflux operon in Escherichia coli. J. Bacteriol. 187:5500-5503.

187. Woolridge, D. P., J. D. Martinez, D. E. Stringer, and E. W. Gerner. 1999. Characterization of a novel spermidine/spermine acetyltransferase, BltD, from Bacillus subtilis. Biochem. J. 340 (Pt 3):753-758.
188. Woolridge, D. P., N. Vazquez-Laslop, P. N. Markham, M. S. Chevalier, E. W. Gerner, and A. A. Neyfakh. 1997. Efflux of the natural polyamine spermidine facilitated by the Bacillus subtilis multidrug transporter Blt. J. Biol. Chem. 272:8864-8866.
189. Xiong, A., A. Gottman, C. Park, M. Baetens, S. Pandza, and A. Matin. 2000. The EmrR protein represses the Escherichia coli emrRAB multidrug resistance operon by directly binding to its promoter region. Antimicrob. Agents Chemother. 44:2905-2907.
190. Zaidi, A. H., P. J. Bakkes, J. Lubelski, H. Agustiandari, O. P. Kuipers, and A. J. Driessen. 2008. The ABC-type multidrug resistance transporter LmrCD is responsible for an extrusion-based mechanism of bile acid resistance in Lactococcus lactis. J. Bacteriol. 190:7357-7366.
191. Zgurskaya, H. I. and H. Nikaido. 1999. Bypassing the periplasm: reconstitution of the AcrAB multidrug efflux pump of Escherichia coli. Proc. Natl. Acad. Sci. U. S. A 96:7190-7195.
192. Zheleznova, E. E., P. N. Markham, A. A. Neyfakh, and R. G. Brennan. 1999. Structural basis of multidrug recognition by BmrR, a transcription activator of a multidrug transporter. Cell 96:353-362.